PRODUKSI SAGU PALM (*Metroxylon sagu rottb)* RESISTAN TIPE III DENGAN METODE HIDROLISIS ASAM-AUTOKLAF SERTA KARAKTERISASI FISIKOKIMIANYA

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ABSTRAK

Pati sagu adalah salah satu jenis pati yang tinggi kandungan amilosa dan amilopektin. Indonesia merupakan salah satu pusat distributor terbesar pati sagu. Sifat dasar pati yang mudah tergelatinisasi membuat penggunaan pasti sagu sangat terbatas dalam produksi makanan. Dalam penelitian ini, pati resisten (RS) diproduksi menggunakan variasi waktu hidrolisis dan konsentrasi asam sitrat dengan menggunakan metode hidrolisis asam dan hidrolisis asam yang diikuti dengan metode autoklaf. Variasi waktu hidrolisis tidak mempengaruhi produksi pati resisten. Karakterisasi dari RS dibandingkan dengan pati sagu murni, dan sagu modifikasi lainnya. Kandungan amilosa menurun setelah dihidrolisis dengan air destilasi dan hidrolisis asam, tetapi meningkat saat dihidrolisis dengan asam yang diikuti proses autoklaf. Kandungan lemak dan protein menurun setelah proses hidrolisis tetapi kandungan serat meningkat, dan nilai serat tertinggi saat menggunakan metode autoklaf. Sampel RS memiliki struktur paling padat saat diukur dengan SEM. Nilai absorbansi spektra UV menurun setelah hidrolisis asam dan meningkat setelah dihidrolisis oleh air destilasi dan menggunakan proses autoklaf. Viskositas, daya kembang dan daya ikat air menurun dibandingkan pati sagu asli dan nilai terendah didapat saat menggunakan metode autoklaf. Emulsi minyak dalam air juga dianalisis dengan menggunakan campuran RS dan kasein yang dibandingkan juga emulsi dari campuran RS dan protein murni dari kedelai (SPI). Selain itu, hylon VII juga dibuat campuran dalam emulsi untuk dibandingkan dengan RS. Viskositas emulsi yang terbuat dari RS+kasein lebih rendah dari pada emulsi yang terbuat dari RS+SPI. Nilai kapasitas emulsi dan stabilitas emulsi lebih bagus saat menggunakan emulsi campuran dari RS-SPI dari pada RS+kasein. Nilai kapasitas emulsi paling besar yang terbuat dari RS+kasein adalah 5.67% (3.75% kasein+ 3.75RS + 7.5% minyak ikan) sedangkan nilai kapasitas emulsi yang terbuat dari RS+SPI sebesar 11.33% (5% SPI + 5% RS + 5% minyak ikan). Selama proses waktu penyimpanan emulsi, nilai peroksida dan anisidin terendah yaitu emulsi yang terbuat dari campuran RS+SPI dan RS-kasein terbuat dari 5% emulsifier (kasein atau SPI) + 5% RS + 5% minyak ikan.

Keywords: pati sagu, metode hidrolisis asam-autoklaf, pati resisten, emulsi minyak ikan, SPI, kasein.

EFFECT OF SiO_2/Al_2O_3 RATIO ON SYNTHESIS ZSM-5 AND ITS CATALYTIC ACTIVITY FOR ESTERIFICATION REACTION

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ABSTRACT

ZSM-5 with different SiO_2/Al_2O_3 molar ratios i.e. 25, 50, 75 and 100 w ere synthesized from kaolin without treatment and ludox as alumina and silica source. The solids were characterized using X-ray diffraction (XRD), infrared spectroscopy (IR), scanning electron microscopy (SEM), and pyridine adsorption techniques. XRD and IR results showed that SiO_2/Al_2O_3 molar ratio effect on the phase and crystallinity of ZSM-5. The morphology and particle size showed similar results, which are joined to form a spherical agglomeration with particle size of about 1-2 μm, as confirmed by SEM. Pyridine adsorption data showed all samples of ZSM-5 have both Lewis and Brønsted acid sites. The catalytic activity of ZSM-5 catalyst were studied in the esterification of kemiri sunan oil. The amount of free fatty acid conversion about 57,95% and the reaction reached equilibrium after 15 minutes.

Keywords: ZSM-5, SiO_2/Al_2O_3 ratio, acidity, esterification reaction

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ANOVA	Analysis of variance
AV	Anisidine value
°C	Degree celsius
cP	Centipoise
EC	Emulsion capacity
ES	Emulsion stability
g	Gram
h	Hour
HCl	Hydrochloric acid
Kg	Kilogram
L	Liter
Min	Minutes
ml	Millilitre
N	Normality
NaOH	Sodium hydroxide
%	Percent
pH	power of hydrogen ion
PV	Peroxide value
RS	Resistant starch
RVA	Rapid visco analyser
SD	Standard deviation
Sec	Second
SEM	Scanning electron microscopy
SPI	Soy protein isolate
UV	ultraviolet
V	Volume
w/v	Weight/volume
w/w	Weight/weight
WHC	Water holding capacity

CHAPTER 1
INTRODUCTION

1.1 Background

Sago starch is extract of the sago palm (*Metroxylon sago rottb*).Starch is highlycollected in the trunk of the sago palm, approximately 250 kg/dry weight plant. In Southeast Asia, It has been considered as one of the important socioeconomic crops, whereby produce 60 milliontones of sago starch annually (Singhalet al., 2008; Ahmad et al., 1999).For a long time, sago starch is used in the food industries for production of traditional foods as sago flour, sago pearl or functional materials (Abdorrezaet al., 2012; Mohamed. et al., 2008). Like other basic starches,characteristics of native sago starch arehigh viscosity, high clarity, low thermal stability, susceptible to acid condition, easily to molded (weak bodied) and gelatinization (Wattanachant et al., 2003; Adzahan, 2002). Besides that, native sago starch undergoes largely break during heating and shearing processes, and alsoretrogradation. Thus, it forms long cohesive gel (Karim et al., 2008).In order to overcome the inherent shortage of native sago starch andimprove its quality for novel food application, native sago starch needs modification.

Resistant starch (RS) is one of the modified products and is resistant to hydrolyze by α-amylase. RS cannot be hydrolyzed in the small intestine, but fermented in the large intestine by colonic flora, and its product consists of short chain fatty acids that enhance health of human digestion.RS can be a substrate for growing of health microorganism and thus can be considered as prebiotics (Ozturk, 2011; Wang, et al., 1999). Besides that, RS can improve the lipid and cholesterol metabolism, so that it can manage glycemic index, diabetes, cholesterol capacity and obesity (Sajilata, Singhal and Kulkarni, 2006). Lopez et al. (2001) has also reported that RS improves the absorption some of minerals in the ileum. Some physicochemical properties of RS are low water holding capacity, bland flavor, improves expansion and crispness in food applications (Waring, 1998).

RS is classified to type I (inaccessible starch in a cellular matrix), type II (native uncooked starch granules that form crystalline, and make them difficult to hydrolysis), type III (retrograded starch, which be formed in cooked), type IV (chemical modified starches) (Shamai, K et al., 2003; Aparicio et al., 2005).Nowadays, the scientists interest of RS formation especially utilization of RS in food production. RS has stability in heating processing and also contains high nutritions. RS type III is generated by combination of the gelatinization-retrogradation process. Gelatinization is interference of the granular structure by heating starch with over water, while retro-gradation is a slow recrystallization of starch main component (amylose and amylopectin) by cooling or dehydration. Initially, starch is heated at fix temperature, it will form starch gel. After cooling, the starch gel will affect crystalline structure. During retrogradation process, amylose is re-arrangement, which causes strong crystallization, finally RS type III is formed. Certain factors influence RS type III formation, including amylose content and chain length, autoclaving temperature, storage time and temperature of starch gel (Huai& Li, 2009).

Lintnerized (partial acid hydrolysis) is one of ways for RS type III formation.Lintnerized starch is obtained by mild acid hydrolysis of α-1,4 and α-1,6 glycosides linkages from amylose and amylopectin. This method increases crystalline content, which becomes resistant by enzymatic hydrolysis. Shin Sanglck et al. (2004) investigated that resistant tuber starch by lintnerization method reached 22.7%.Aparicio et al. (2005) also has investigated that resistant banana starch is obtained 16% from this method, and then autoclaved, itshows a lower solubility in water than native starch and RS value is higher than only lintnerized treatment. Besides that, Aparicio's research (2005) has showed that resistant starch prepared by lintnerized-autoclaved contained slowly digestible carbohydrate.It indicates that this method has potential for the development of food applications. Whereby,RS type III formation by lintnerized methods is influenced by strength of acid, incubation time and temperature (Onyango et al., 2006; Koksel et al., 2007; Zhao and Lin, 2009).Certain researches applying lintnerized method usually use hydrochloric acid with high adequate concentration. Further, it is applied in food industry. As known, hydrochloric is

toxic and strong acid. It is hoped to decrease application of hydrochloric acid in food industry. Zhao and Yang (2009) suggested that utilization citric acid to de-branch on RS type III formation is better than hydrochloric acid and acetic acid. They have reported that retrograded high amylose maize using citric acid at room temperature shows significantly increase RS yield. Present study is to evaluate optimization of the formation of RS type III of sago starch and its functionto enhance nutrient value then can be applied for food industry-rich healthy ingredient. A lintnerizedmethod which will use is acid citric. It is nutritionally harmless, compared to other derivatization.

On the other hand, fish oil, which is rich source of omega-3polynsaturated fatty acids and very susceptible to lipid oxidation is another important functional compound that is used in food applications, such as fish oil emulsion. Fish oil emulsion needs mixtures of protein and carbohydrate to form the Millard reaction products by increasing emulsifying properties and oxidative stability of fish oil emulsions (Kato, 2002; Morris et al., 2004; Anal et al., 2012). RS which has characteristics such as less solubility, high crystalinity and stability in high process temperature can be used in combination with proteins to prepare fish oil emulsion to keep oxidative stability of fish oil. Nasrin et al., (2014) reported that oil in water emulsions prepared by mixture of culled banana pulp resistant starch and soy protein isolate (SPI) were the most stable than mixture of Hylon VII and SPI or using SPI only, resulting the lowest amount of peroxide value and anisidine value as a total oxidation value which were occurred for storage times. In this study, RS production is utilized as mixture of fish oil emulsion and also by comparing using emulsifiers SPI as protein from vegetable and casein as protein from animal. Britten and Giroux (1991) found that emulsions stabilized with casein showed a better stability than those stabilized by whey proteins. Besides that, Mulvihill and Murphy (1991) reported that emulsions were more stable when using casein as emulsifier than that of sodium caseinate.

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1.2 Statement of the Problems

Limited reports are available on theresistant starch type III from sago (*Metroxylon sago rottb*), whereas, modification sago starch is needed to improve quality and its

nutrient, especially to increase its functional ingredients in food productions. To the best our knowledge, present study will use lintnerized-autoclaved method to optimize RS type III. Besides that, RS production will investigated the influence toward fish oil emulsion by comparison using emulsifier from SPI and casein because known RS has characteristics: less solubility, high crystalinity and stability in high process temperature which can stabilize lipid oxidation of fish oil.

1.3 Objectives of the Research

Overall objective of this study is to explore benefit sago starch by producing RS type III, to increase economical value of sago starch,to give the alternative food material-rich dietary fiber, and also to formulate fish oil emulsion by using casein and SPI as emulsifier.

1.3.1 Specific objectives

1. To optimize the lintnerized-autoclaved process to get high RS type III, focus on concentration of acid citric, and time of hydrolysis.
2. To enhance the physicochemical properties of sago starch by comparing physicochemical properties of lintnerized-autoclaved sample with native sago starch, lintnerized starch and hydrolyzed starch by distilled water.
3. To investigate the effect of RS with proteins as emulsifier to produce fish oil emulsion and also to compare those emulsions also using mixture of Hylon VII and emulsifier and using only emulsifier.

1.4 Scope

This study consists of three stages. In the first stage, sago starch is hydrolyzed by variation concentration of acid citric and time of hydrolysis then autoclaved-cooled (three times cycles) by suitable temperature, and then measured RS value of each sample from lintnerized starch and lintnerized-autoclaved starch. Sample that has the highest value of these variations will analyze further. For comparison, sago starch is also hydrolyzed by distilled water. So that this study will have four variations of samples for analysis further including native sago starch,

hydrolyzedstarch by distilled water, lintnerized starch and lintnerized-autoclaved starch.Second, these samples will be analysed for chemical-physical composition: moisture, protein, lipids, ash, carbohydrate, amylose, pasting properties, solubility, swelling power, water holding capacity,scanning electron microscopy and UV/visible analysis.Third, RS sample will be applied as mixture of fish oil emulsions after known that RS sample give good properties for this application. The emulsions will be analyzed emulsion capacity, emulsion stability, peroxide value and anisidine value, compared with emulsions mixture of Hylon VII as native starch rich amylose and emulsifier. Besides that, the emulsifiers used in this research were SPI as protein from vegetable and casein as protein from animals, further this case can compare the effect toward emulsions using different emulsifier.

1.5 Overall experimental plan

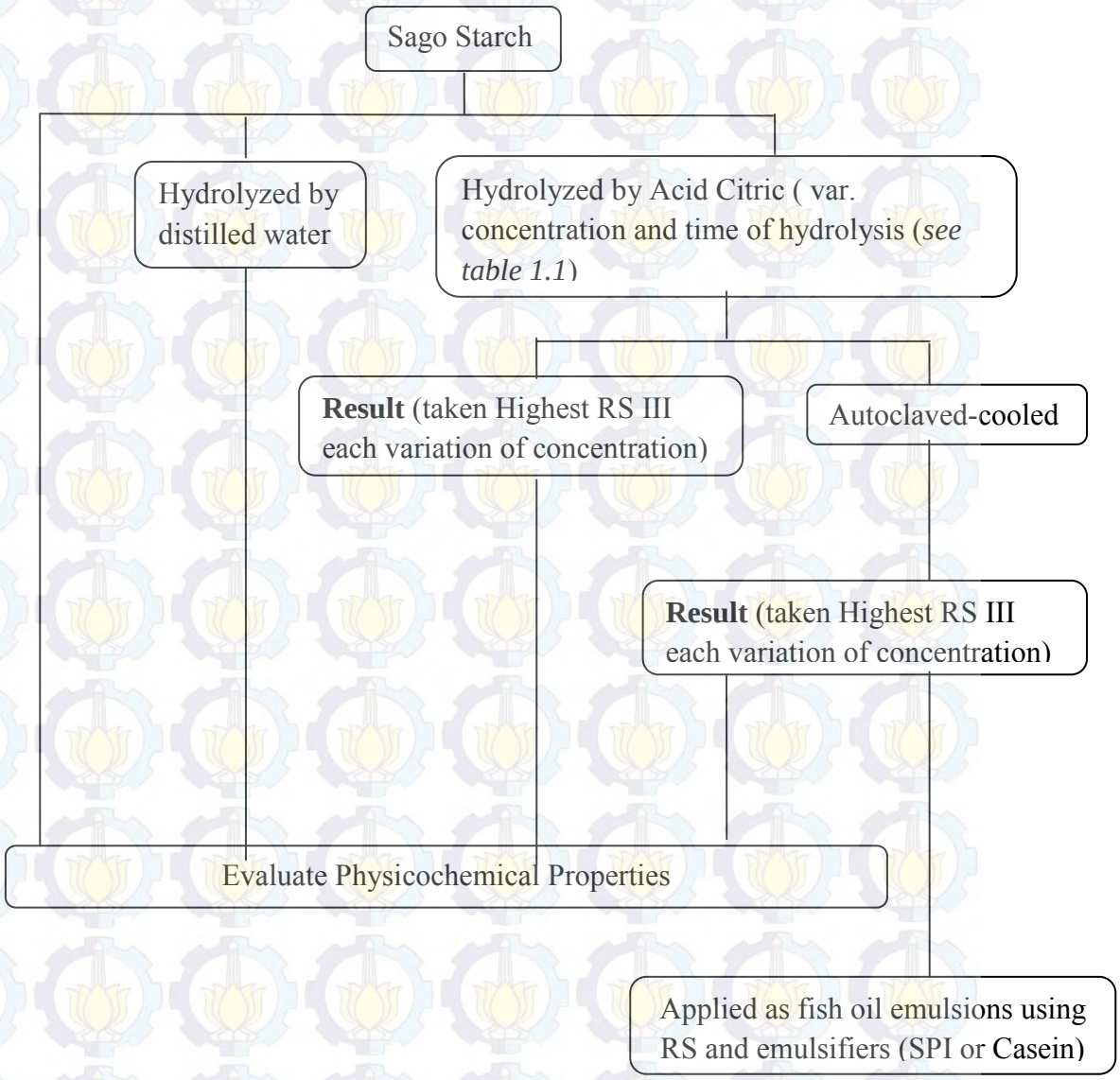

Figure 1.1 overall experimental plans

Table 1.1 Variation of Concentration of Acid Citric and Time of Hydrolysis; RS contents

time of hydrolysis (h)	Concentration of Acid (N)	RS value (%)	
		Lintnerized-autoclaved	Lintnerized
3	1		
	1.5		
	2		
6	1		
	1.5		
	2		
12	1		
	1.5		
	2		

CHAPTER 2
LITERATURE REVIEW

2.1 Sago Palm (*Metroxylonsagurottb*)

Sago palm is shown in Figure 2.1. Sago palm is the old tropical plant which tolerate in wet condition. Its tall is 6-14 m, sago palm converts its nutrients into starch and the the trunk is filled before flowering. Figure 2.2 exhibit extraction of sago starch which is contained in the trunk of sago palm. The productivity of sago starch is higher up to 4 times than that of starch from paddy. Sago has still low attention for main food if compared by rice and cassava, especially in Asia. Indonesia distributes approximately 96% of sago in the world (2.250.000 Ha). Taxonomy of sago palm is shown in Table 2.1.

Figure 2.1 Sago Palm (*Metroxylonsagurottb*)

A B C

Figure 2.2 Sago starch extraction by traditional method. A) Extract is scraped, separated from its peels; B) water is added and mixed them; C) Wet starch is collected.

(Karim et al., 2008).

Table 2.1 Taxonomy of Sago Palm (Source: IT IS report, 2014)

Specification	Name
Kingdom	Plantae
Division	Tracheophyta
Subdivision	Spermatophytina
Class	Magnoliopsida
Order	Arecales
Family	Arecaceae
Genus	*MetroxylonRottb*
Species	*MetroxylonsaguRottb*

2.2 Starch

Starch is one of the most nature carbohydrates from plantwhich is rich with two polysaccharides, including amylose and amylopectin. Chemically, starch is linked with α-D-(1-4) and or α-D-(1-6) bonding. Amylose and amylopectin link by hydrogen bonding.Starches from various sources have different ratio of amylose and amylopectin which affect on quality of food production. High amylose starch

is utilized to reduce oil absorption fried food, it forms strong film. Thus, high amylose can enhance crispiness. Amylose structure is shown inFigure 2.3 which has a linear structure, degree ofpolymerization around 6000 and a molecular weight of 105 to 106 g/mol, besides that the chains of amylose can easily form helix structure both single and double structure. Characteristics of amylose are also insoluble in water, the structure more compact and resistant to digest by enzymes. Therefore amylose can be used to produce resistant starch. In contrast, Amylopectin which is exhibited in Figure 2.4 has highly branches structure and soluble in water, thus it is so easy to digest by enzymes. Amylopectin has molecular mass 107 to 109 g/mol, and the degree of polymerization approximately 2 million. (Thompson et al., 2002; Sajilata, et al., 2006; Singh, 2012).

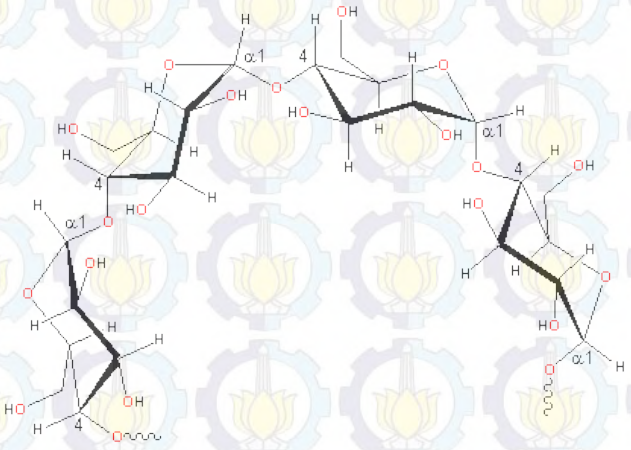

Figure 2.3 Amylose structure

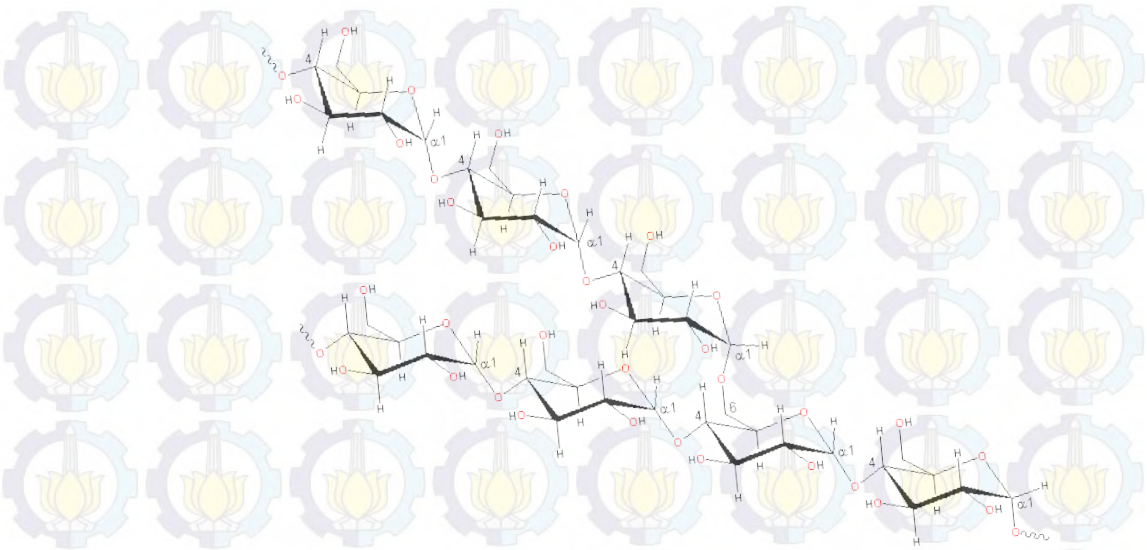

Figure 2.4 Amylopectin structure

2.3 Sago Starch

The size of granule of sago starch is around 10-50µm. Sago starch is commonly used as functional ingredient in food production, such as thickener, stabilizer, and gelling agent.Its physicochemical characteristics such as molecular weight, viscosity, ratio of amylose and amylopectin, swelling power, gelatinization and retrogradation are the most important thing when determining sago starch is used in food industry.Lee et al., (2002) found that sago starch paste is softer than the paste of cereal starches. By comparison with other starches, sago starch gel is firm, because of higher cohesiveness. Besides that sago starch is resistant to enzymes when it was compared to cereal starch (Haska et al., 1992).

Table 2.2 Chemical and physical Properties of Sago Starch

Component	Value
Moisture	10.6 -20.0 %
Ash	0.06-0.43 %
Crude fat	0.10-0.13 %
Fiber	0.26-0.32 %

Crude protein	0.19-0.25 %
Amylose	41 %
Amylopectin	59 %
Molecular weight of amylose	1.41 $x10^6$ – 2.23$x10^6$
Molecular weight of amylopectin	6.70 $x10^6$ – 9.23$x10^6$
Viscosity of amylose	310-460 ml/g
Viscosity of amylopectin	6.70$x10^6$-9.23$x10^6$ ml/g
Gelatinization temperature	69.5-70.2^oC

(Source: Sim et al., 1991; Ahmad et al., 1999; Nisa et al, 2013).

2.4Swelling Power of Starch

The swelling occurs when starch is added over water and heated it. The starch granule will swell and its volume is over. Double helic structure of starch will break because hydrogen bond of starch is replaced by hydrogen bond of water which makes weaker interaction inside of starch. This interaction is affected by ratio of amylose and amylopectin that directly relates with structure of amorphous and crystalline of starch (Tester and Karkalas, 1996; Hoover ., 2001). Amylose-lipid complex and sodium chloride also affect on swelling of starch which can inhibit interaction between granulastarches. Maximum value of swelling power of sago starch is 31% of sago starch.The swelling decrease when sago starch concentration increase.

2.5 Gelatinization of Starch

When starch suspensions in water are heated above the gelatinization temperature, swelling power will occur a long time and change the starch structure, but granules still hold their identity(Hung et al., 2001). The changing of its structure are release small molecules weight polymers, such as amylose, then loss the crystalline and birefringence, and finally sago starch will be soluble. During gelatinization, starch granules swell and form gel particles. Generally, the swollen granules are enriched in amylopectin, while amylose spreads out of the swollen

granules and make up the continuous phase outside the granules (Hermansson and Svegmark, 1996).

2.6 Retrogradation of Starch

Retrogradation occurs upon cooling and storage of gelatinized starch. Retrogradation loses its properties depending on the storage time and temperature. Retrogradation is also called one of cause of food quality deterioration (Karim et al., 2000). However, retrogradation is promoted to modify the structural, mechanical or organoleptic properties of certain starches based products. Starch retrogradation has been defined as the process which occurs when molecular chains in gelatinized starches begin to re-associate. During retrogradation, amylose forms double helical whereas amylopectin crystallization occurs by re-association of the outermost short branches (Ring et al., 1987). The retrogradation of amylopectin is influenced starch source, concentration, storage temperature and other component (Slade et al., 1987). Crystalline ability in starch gels is formed of the amylose fraction. Thus,retrograded amylose is a important indigestible starch fraction which is stable and melts around120^{o}C (Sievert and Pomeranz, 1989).

2.7Classifications of Starch

Table 2.3 Starches are classified into three typesbased on the action of enzymes, (adapted from Sajilataet al., 2006)

No	Clasification	Description
1	Rapidly digestible starch	- Amorphous and dispersed starch - Found in starchy cooked by moist heat - Example : bread and potatoes
2	Slowly digestible starch	- Physically inaccessible amorphous starch - Completely but so slowly digested in small intestine - Example : cereals
3	Resistant starch	- fraction of dietary starch - escapes digestion in the small intestine - Resistant to hydrolysis by exhaustive α-amylase and pullulanase treatment.

Table 2.4 Native starches are classified into four typesbased on X-ray diffraction, (adapted from Wu and Sarko, 1978)

No	Classifications	Description
1	Type A	- Has amylopectin of chain lengths of 23 to 29 glucose units. - Amylopectin contains 4 water molecules per 12 glucose residues - The hydrogen bonding of amylopectin form outer double helical structure. - Linear chain of amylose has densely packed double helices - Found in cereals
2	Type B	- Has amylopectin of chain length of 30 to 44 glucose units. - Contains 36 molecules per 12 glucose residues - Loosely packed double helices - Found in potato and banana.
3	Type C	- A combination of type A and type B - Has amylopectin of chain length of 26-29 glucose molecules - Found in beans
4	Type V	- Has single helical - Occurs in swollen granules - Initiated in amylose complex with lipid or other agent.

This bellow shows X-ray diffraction diagrams of these starches,

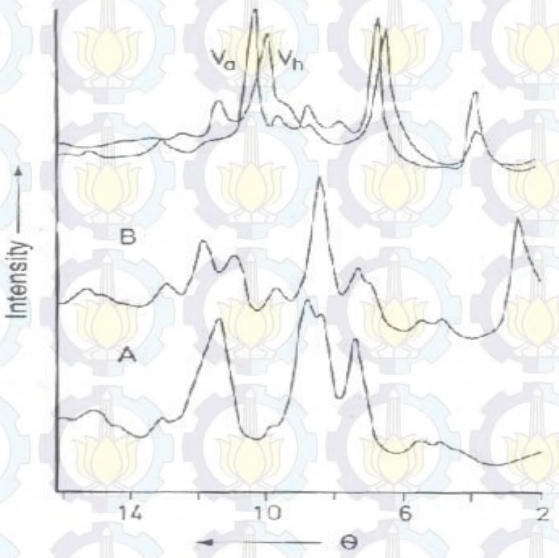

Figure 2.5 XRD pattern of starches: type A (cereal), type B (legumes), and type V (swollen starch, V_a :water free, V_h : hydrated)

(Galliard, 1987).

2.8 Resistant Starch

Resistant starch (RS) is defined as the fraction of starch, which cannot be digested in the small intestine and fermented in colon by bacterial flora. RS has been an increased interest in the nutritional food. Not only it can decrease caloric content but also has a similar physiological effect as dietary fiber. RS is classified into four types:

1. RS type I

$RS_1$is in a inaccessible form such as grains and seeds. RS_1 is heat stable in the normal cooking. RS_1 is found in undamaged cell wall of plant. Amilase cannot degrade RS_1 because RS_1has hardcomponents of plant such as cellulose, hemicelluloses, and lignin.

2. RS type II

RS_2has compact structure which can limit the enzymes accessibility. It is resulted from the physical structure of the raw materials or uncooked starches, such as potato, banana and high-amylose maize which have crystallinitysothatmaking them seldom to behydrolyzed. However, the enzymes resistance of these starches willdecrease after heatingwith excess water. Thus RS_2has limitation on using of food productions.

3. RS type III

RS_3 productions are made by processing of gelatinization and retrogradation. Gelatinization process can break down the structure of granular by heating and presence of water, whereas retrogradation process is recrystallization process of amylose and amylopectin process by cooling and dehydration treatment. Combination of these processes will form double helic structure which is stabilized by hydrogen bonding. Because of that, RS_3 is stable in high termal.

4. RS type IV

$RS_4$is new chemical bonds not only α-(1-4) or α-(1-6) bonding. Certain chemical modifications to improve the physicochemical properties of sago starch have been studied, such as acetylation, hydroxypropylation and cross-linking. Characteristic of RS_4 are more resistant to shear and acidic condition. RS_4 is reaction of starch and chemical reagents that can form ether or ester inter molecular linkages between hydroxyl groups of starch molecules.

2.9 Factors Influence Resistant Starch

Certain factors give influence for RS yield :

1. Amylose

Amylose can form complex with lipid which has lower digestibility (Tester et al., 2006; Singh et al., 2010). Amylose-lipid complex reduces contact enzymes and substrate, thus it gives limited digestibility compared to free amylose.

2. Sugar

Englyst et al., (2003) reported that addition of sugars influences the degree of starch gelatinization. It can increase gelatinization temperature. Interaction sugar molecule and starch will change matrix of gelatinized starch, and it can decrease formation of RS.

3. Protein

Protein also reduces the rate of enzymatic hydrolysis by covering the adsorption site of the starch. During autoclaving and cooling cycles of potato starch mixing with protein (albumin) decrease RS contents. Physically, protein network in cereal limits the accessibility of starch to amylase. Thus, it can increase resistance level toward amylase.

4. Ions

Ions such as K^+ and Ca^{2+}also influence on RS formation. These ions inhibit the formation of hydrogen bonds between amylose and amylopectin chains. Hence, it can decrease RS value (Hoebler et al., 1999).

5. Amylase Inhibitor

Compounds of amylase inhibitor such as tannin acid, lectin, polyphenols can inhibit amylase activity. They can also decrease glycemix index (Thompson et al., 1984)

6. Type, Granular Shape and Crystallinity of Starch

Different type of starch also influences the formation of RS. RS_1 (e.g cereals) has highest resistant in digestion than RS_2 (e.g banana and potato). Granular shape, surface characteristics also influence in RS formation. Small size of starch granules are easilyhydrolyzed by enzyme than that of bigger size (Svihus et al 2005; Noda et al., 2008; Parada, 2009). Crystalinity of starch depends on the chain lengths to build amylopectin lattice, the density of granules and water contents (Wu and Sarko, 1978). The crystalline of A and B hasthe same type of double helices conformation but having different water contents. Break down of plant cell increase interaction of enzyme and then will reduce RS value.

7. Linearization of amylopectin

Linearization of amylopectin also gives impact for RS formation. Linearization occurs during the long low temperature in present of certain organic acid, for example bread productions bake with added lactic acid. Berry (1986) has reported that RS formation increases during wet-autoclave.

2.10 Resistant StarchProcessing

Starchy food processing usually uses heat treatments in presence of water. This treatment produces edible product, increases the nutritive value and result desirable flavor and texture (Miller, 1988). RS formation is influenced by

changing of moisture, temperature, and duration of heating-cooling cycles (Perera et al., 2010). This below will explain food processing technique.

1. Milling

Milling is a high shear process. When starch granules are milled, their crystalline regions are damaged (Devi et al., 2009). The disruption of granule structure during miling increases the susceptibility to enzyme degradation (Lehmann et al, 2007; Mishra et al., 2009).

2. Cooking

Cooking increases the rate of starch hydrolysis by gelatinizing the starch and making it more easily to be attacked by enzymes (Bornet et al., 1989). Cooking process is done by using over water in high temperature. This treatment can disturb crystalline structure. RS formation increase by steam treatment.

3. Heating-cooling

Heating-cooling process improves the textural properties and also products of this process reduce digestibility of starch by enzymes (Whalen et al., 2000). When the starch gels are cooled, molecules of gelatinized starch begin to retrograde, and increase thecrytallinity of their structures. Hence, starch becomes less susceptible to be hydrolyzed byenzymes such as α-amylase (Oates, 1997; Buleon et al., 1998).Farhat et al., (2000) also reported Storage temperature affected on retrogradation of starch.Gelatinized starch which is stored alternately and repeatedly at cold temperature (4^oC) and room temperature (30^oC) increase RS formation. Structure of amylose and amylopectin re-arrange to form crystalline structure. It can reduce digestibility or hydrolysis by enzymes or chemically(Park et al., 2009).

4. Extrusion

Extrusion is a thermal process using high heat, high pressure and shear forces to uncooked materials. There are some differences about effect extrusion on RS value. Some researches reported that process of extrusion decrease RS value

(1997; Unluet al., 1998;Farhat et al., 2001; Wolf, 2010) but Chanvrier et al., (2007) andHuth et al., (2000) reported that process of extrusion increase in RS value. Kim et al., (2006) also reported that RS contents of wheat flour increase from ranging 0.52% to 2.65% after extrusion.

2.11Resistant Starch Production

Resistant starch can be produce from certain methods.

1. Heat Treatment

Like previous explanation that heat treatment is done by heating with extra water above gelatinization temperature then dehydrated. Optimum result which is done using heat treatment is temperature of 120^{o}C for 20 min. Garcia et al., (1999) studied that heat treatment procedure consist of gelanitinization and retrogradation process. After getting retrograded starch, that sample is dried at 60^{o}C then milled it.

2. Acid Modification

Acid modification is one of chemical method used to prepare RS productions. Lintnerization or partial acid hydrolysis is hydrolysis by using mild acid at below gelatinization temperature of sample. After that process, sample usually is neutralized until neutral pH. Some factors which affect on acid modification are concentration of acid, time of reaction and temperature of hydrolysis. This method can change the properties of starch but it does not change the granula structure of starch. Partial acid hydrolysis improves the solubility adn gel strength of starch but it decreases it viscosity.Lintnerized process which is followed by autoclaving-cooling treatment can increase RS formation.

3. Enzymatic Treatment

Enzymatic treatment can be used to debrach starch structure, such as pullulanase. This enzyme can cleave α-1,6 linkages in amylopectin and other polysaccharides. Debraching of amylopectin increases aggregation, and form crystalline structure. This treatment also increase RS yield (Lin, and Chang, 2006). Zhao et al. (2009)

have reported that maize starch which ishydrolyzed by pullulanase for 12 h, then two autoclaving-cooling cycles increases RS formation.

4. Chemical Modification

RS is also produced by chemical modifications such as acetylation, hydroxypropylation and cross-linking. Several reagents that used for this treatment are epychlorohydrin, phosphoryl chloride, sodium trimetaphosphate, sodium tripolyphosphate, and a mixture of adipic acid and acetic anhydride (Lim et al., 1993; Ratnayake et al., 2008; Carmona et al., 2009).

2.12 Resistant Starch as Dietary Fiber

Dietary fiber is carbohydrates which are resistant to digest in human small intestine but it are fermented in colon (large intestine). Analogous dietary fiber is material which has the similar properties of fiber. Examples of analogous dietary fiber are modified cellulose, resistant starch which has interested the scientists to explore deeply (AACC, 2001). On the other hand, Peres et al.,(2008) andCharalampopoulus et al., (2002) reported that resistant starch has better texture, mounthfeel than pure fibers (grains, or fruit fibers). Thus, resistant starch can be raw material of food production, such as bread, cake, and pasta. Besides that, RS also is health food especially providing energy to bacteria in large intestine which can increase healthy fermentation.

2.13 RS as an encapsulating agent in food production

An encapsulating agent is a material from various biopolymeric materials such as protein, carbohydrates and lipids which are used to coat another material, usually a sensitive compound and its functions are to minimize oxidation, enhance shelf life and preserve nutrition (Anal et al., 2007 and Acosta, 2009). Resistant starch (RS), one of derivative of polysaccharide, has been used as an encapsulant material to protect from heat treatment and enhance the shelf life of sensitive compounds because RS has less solubility, high crystallinity, and stability in high processing of temperature. Chung et al., (2010) reported that RS made from Hylon VII was mixed with sodium casein to produce fish oil microcapsule. On the

other hand, Sultana et al., (2000) obtained that Hi-maize starch was used to encapsulate *Lactobacillus acidophilus*and *Bifidobacteriumspp,* increasing the their survival in yogurt.

2.14 Emulsion

An emulsion, one of encapsulation techniques, is mixture of two liquids that hold their obvious characteristics, considered immiscible liquids, such as mixtures of fat and water. In oil in water emulsion, oil is defined as the dispersed phase and water is continuous phase. Examples of oil in water emulsion are mayonnaise, cream and milk. Adding more of the continuous phase will thin an emulsion whereas more of the dispersed phase will thicken an emulsion. Only mixture of oil and water cannot mix well so that shearing powers such as shaking, homogenizer are needed in emulsion process to break down the dispersed phase then trapped it into continuous phase. To get smaller of dispersed phase, it needs more shearing power. It also makes emulsion more stable. But in fact, it will be unstable again by nature. Thus, emulsion system needs emulsifiers to make stable a long time.

There are two basic types of emulsifiers: amino acid chains and phospholipids. Amino acid chains will link together, forming proteins. Some amino acid chains have also hydrophobic and hydrophilic part, such as casein. The second form of emulsifiers are phospholipids such as lecithin, found in soy, classified as a surfactant, meaning it has a water friendly head and a fat friendly tail. Lecithin also has a positively charged tail which makes it anhighly effective emulsifier for fat in water emulsion.

Emulsion is affected by some factors, including oxygen, oil quality, metal ion, temperature, emulsifiers, pH and antioxidants. (1) Oxygen can oxidize lipid rapidly, it is highly soluble in fat. Thus, sensitive compound such as fish oil containing omega-3 should be kept from air. The production can be done under vacuum condition. (2) Oil quality also affects to emulsion, relating with oxidation level. Good oil contains peroxide value<0.5 meq/kg. Quality of oil is very important because it is to get good emulsion. (3) Metal ions, such as iron and copper are considered the most oxidation catalyst in food product. (4)

Temperature; high temperature oxidize lipid rapidly. Recommended, during production and storage, the emulsion should be kept in cold temperature. (5) Emulsifier and pH; emulsifiers can increase the stability of emulsions, it decrease surface tension between oil and water. Level of pH also can affect on emulsion, so it should adjust pH appropriately. (6) Antioxidants have a great influence on oxidative properties of fat. It can grab free ions and oxygen (Mei et al., 1999; Mozyraityle et al., 2006).

2.15 Casein

There are two distinct types of proteins in milk, casein and whey. Caseins make up over 80% of the total protein content. Casein is divided into five groups αs_1-, αs_2-, β-, κ- and γ caseins. The amino acids in casein have hydrophobic and hydrophilic regionswhich acts as stabilizers of emulsions. Caseins are disordered and become hydrophobic, which support their rapid absorption during processing of emulsion. Casein easilycoagulates at the isoelectric point (pH 4.6) (Southward, 1985). Physicochemical characteristics of the caseins are exhibited in Table 2.5. The caseins are hydrophobic protein, but the hydrophobic residues are not free distributed along the polypeptides. Casein has also many polar residues such as phosposeryl residues (Mepham et al., 1982). Caseins have a amphipathic properties which make it good for emulsifiers materials.

Table 2.5 Some physicochemical properties of caseins

Property	Caseins			
	αs_2-	αs_2-	β-	κ-
Molecular weight (Da)	23.614	25.230	23.983	19.023
Residues/molecule (Kj/residue):				
Amino acids	199	207	209	169
Proline	17	10	35	20
Cysteine	0	2	0	2
Disulphide	0	1	0	1
Phosphoserine	8	11	5	1
Isoionic point	4.96	5.19	5.19	5.43
Charge at pH 6.6	-21.9	-12.2	13.8	-3.0
Hydrophobicity	4.9	4.7	5.6	5.1

2.16 Soy protein Isolate

Soy isolate protein (SPI) contains 90% protein, the major components are glycinin and β-conglycinin with the molecular weight 320-375 kD and 140-210 kD respectively. These fractions consist of 34 and 27% of the isolate proteins, respectively. Solubility of SPI in is affected by pH, ionic strength and temperature. Solubility of SPI is high at ends of the pH scale but it is not soluble around its isoelectric point (pH 4.5) (Wolf, 1983; Kinsella, 1979). On the other hand, solubility of SPI increases more than 20% when the temperature is increased up to 50^{o}C (Lee et al., 2003). Heating treatments of SPI dispersions increase viscosity because it candenaturate protein which increase interaction of each protein.

Emulsion capacity (EC) and stability (ES) of soy protein are lowest at the isoelectric points and increase at pH below or above of isoelectric points. EC and ES are also higher for the protein rich β-conglycinin fraction than protein rich with glycinin fraction (Aoki et al., 1980). It relates with properties of hydrophobicity of β-conglycinin fraction. Besides that, emulsion will be better if using high concentration of protein, around 1.25- 1.5 mg/ml. Heating process also influences on SPI properties to prepare good emulsion because heating can increase hydrophobicity of SPI (Santiago et al., 1998).

2.17The Previous Studies

This bellow shows certain previous studies which support this research.

Table 2.5 List of the previous research

Title	Description	Researcher and Years
Resistant starch III from culled banana and its fuctional properties in fish emultion.	• Lintnerized used HCl 1N; 1.5N; 2N at 40^{o}C for 3 h • Amylose content decreased after lintnerization, but increased in lintnerized-autoclaved samples • Increasing RS yield by lintnerization-autoclaving	Nasrin and Anal (2014)

	process. • Viscosity value decreased with increasing the concentration of acid level. • Emulsion made by the mixture of soy protein isolate (SPI) and RS was the most stable than only using SPI or mixture SPI and Hylon VII.	
Resistant starch-rich powders prepared by autoclaving of native and lintnerized banana starch: partial characterization	Containing highest RS formation by lintnerized-autoclaved (1.51% to 19.34%). Lintnerized used 1 M HCl at 35^{o}C for 6 h.	Aparicio et al., (2005)
The impact of couple acid or pullulanasedebranching on the formation of resistant starch from maize starch with autoclaving-cooling cycles.	Increasing RS yield (8.5% to 11% by lintnerized acid citric 0.1 mol/L; T= room temperature for 12 h, followed three autoclaving-cooling cycles.	Zhao and Li (2009)
Slowly digestible cookies prepared from resistant starch-rich lintnerized banana starch.	Increasing RS yield (1.48% to 8.42%) by lintnerized acid citric 0.5 g/L in blender low speed for 2 min, followed by autoclaved-cooling cycles. This result was suggested as slow carbohydrate (based on predicted glycemic index.	Aparico et al., (2006)
Influence of incubation temperature and time on resistant starch type III formation from autoclaved and acid hydrolyzed cassava starch	Highest quantities of RS formation was gotten by autoclaved starch-suspended in 10 mmol/L lactic acid at 60^{o} C for 48 h.	Onyango, Calvin et al., (2006)
Mild hydrolysis of resistant starch from maize	Highest RS yield (from 3.5% to 44.1%) was gotten by hydrolysis in 0.1 M HCl at 35^{o}C for 30 days.	MunSae and Shin (2006)

Physicochemical, thermal and rheological properties of acid-hydrolyzed sago (*Metroxylonsagu*) Starch	• Molecular weight of amylopectin and amylose were decreased after hydrolyzed by HCl 0.14 mol/L for 24 h. • Amylose decreased 5.6 % after hydrolyzed. • Swelling power was decreased and solubility increased by increasing the duration of acid treatment. • Pasting properties was decreased upon increased duration of hydrolysis. • The gelatinization temperature was increased by acid treatment.	Abdorreza et al., (2012)
Effect of debraching and heat treatments on formation and functional properties of RS from high-amylose corn starches	• Molecular weight of samples decreased and RS contents increased with increased debraching time. • RS contents of Hylon VII sample were higher than those of Hylon V samples • The solubility and water binding values of autoclaved sample, autoclaved-debrached sample and autoclaved-cooled sample after debraching were higher than those of their respective native starches. • Autoclaving-storing cycles after debraching caused decreases in peak, breakdown and final viscosity values.	Ozturk, Serpil et al., (2009)
Production of RS from acid-modified amylotype starches with enhanced functional properties	• MW of the samples decreased with increasing hydrolysis time. • Acid-hydrolyzed and autoclaved-stored samples	Ozturk, Serpil et al., (2011)

	increased the emulsion capacity and stability values of albumin	

CHAPTER 3
METHODOLOGY

3.1 Materials

Sago starch was brought from Indonesia, processed by AliniCompany (Figure 3.1). All other chemicals (citric acid, NaOH,sodium maleate buffer, sodium acetate buffer, sulfuric acid, o-dianisidine reagent, iodine, H_2SO_4, HCl, petroleum ether, bromocresol green indicator, methyl red indicator, pancreatic α-amylase, amyloglucosidase, acetic acid, chloroform, potassium iodide, iodine, sodium thiosulphate, casein,hylon VII,para-anisidine, glacial acetic, isooctane, fish oil) used in this research were analytical grade.

Figure 3.1 Sago starch production by AliniCompany, Indonesia

3.2 Methods

1. Lintnerization of Starch

The modified methods of Nasrin et al., (2014) were used to produce lintnerized sago starch. Sago starch was suspended into 1 N; 1.5 N; and 2 N citric acid solution at 1:1.5 (w/v) ratios. Mixtures were heated at 60^{o} C and used variation time of hydrolysis (3h; 6h and 12 h) and then, samples were neutralized with NaOH 10% and washed properly by distilled water.Samples were dried at 40^{o}C for 2 days, cooled down, passed through 100 mesh sieves and stored in dessicators.

2. Distilled Water Hydrolysis of Sago Starch

Water hydrolysis was prepared according to Zhao and Lin's method (Zhao and Lin, 2009) with modification. Sago starch (10 g) is dispersed in 40 ml of distilled water and the mixture is autoclaved at 121^{o} C for 1 h. hydrolysis sample was dried at 40^{o}C for 2 days, cooled down and milled to produce fine particle through 100 mesh sieves and then stored into dessicators.

3. Preparation of Resistant Starch

Samples, includinglintnerized starch and lintnerized-autoclaved starchwere suspended in water (1:10 w/w) and gelatinized at 85^{o} C for 30 min. Samples were autoclaved at 135^{o}C for 30 min, cooled down and store at 4^{o}C for 24 h. Autoclaving-storing treatments were repeated three times at same temperature and time. Samples were dried at 50^{o}C, cooled down, milled and sieved through 100 meshes.

4. Preparation of fish oil emulsion

Mixture starch (using RS or usingHylon VII as native starch rich amylose) and emulsifier (casein or soy protein isolate) based on Table 3.1 were added with water 60^{o}C to get aqueous suspensions (10% total solids, w/w), heated at 100^{o}C, cooled at room temperature and then frozen and lyophilized. Freeze-dried materials were added fish oil and water based on Table 3.1 to obtain 15% w/w emulsions. Each mixture was blended then homogenized. All emulsions were adjusted at pH 7.5, thenanalyzed for emulsion stability, emulsion capacity, viscosity and color value. Besides that, during storage the emulsions were analyzed peroxide value and anisidine value.

Table 3.1 Formulations of fish oil emulsions

Emulsion systems	Compositions (% w/w)					
	Emulsifier	RS	Hylon VII	Fish oil	Water	Total solid
E_1	7.5	0	0	7.5	85	15
E_2	3.75	3.75	0	7.5	85	15
E_3	3.75	0	3.75	7.5	85	15

E_4	10	0	0	5	85	15
E_5	5	5	0	5	85	15
E_6	5	0	5	5	85	15

E_1= 7.5% emulsifier (casein or SPI) + 7.5% fish oil; E_2= 3.75% emulsifier + 3.75% RS + 7.5% fish oil; E_3= 3.75% emulsifier + 3.75% Hylon VII + 7.5% fish oil; E_4= 10% emulsifier + 5% fish oil; E_5= 5% SPI + 5% RS + 5% fish oil; E_6= 5% Hylon VII + 5% fish oil. 85% water in all systems.

5. Analysis of Physicochemical Properties

I. For Lintnerized and lintnerized-autclaved starch samples

1. Resistant Starch

The resistant starch analysis used the methods described by McCleary and Monaghan(2002) with modification. Sample (100 mg) was placed into a centrifuge tube. Sodium maleate buffer 1 M (pH 6.0) containing pancreatic α-amylase (10 mg/ml) and amyloglucosidase (3 U/ml) was added 4 mL. The tube was closed, mixed up on vortex mixer and incubated them in shaking water bath at 37^{o}C for 16 h. The reaction was stopped, added 4 ml ethanol (99%) and followed by centrifugation at 3000 rpm for 10 min. Supernatant was separated, then starch lump was added ethanol (50% v/v) 8 ml, stirred and centrifuged again.Resistant starch is measured by adding 2 ml KOH 2 M, and added 8 ml sodium acetate buffer 1.2 M (pH 3.8) and 0.1 ml of amyloglucosidase (3000 U/ml). The mixture was incubated with continuous shaking at 50^o C for 30 min. The glucose was determined by glucose oxidase assay.Sampel was added glucose oxidase peroxidase solution containing o-dianisidine reagent, and then incubated at 37^{o}C for 30 min. Sulfuric acids 12 N was added 2 ml to stop its reaction. The absorbance was measured by spectrophotometer (Model UV2, Unicam, England) at 540 nm(Bergmeyer and Bernt, 1974).

II. For native starch, hydrolyzed starch by distilled water, lintnerized starch and lintnerized-autoclaved starch samples

1. Amylose

Sample (100 mg) was put into 100 mlerlenmeyer. Ethanol 95% and NaOH 1 N were added 1 mL and 9 mL, respectively. Mixture was heated for 10 min in

boiling water bath, cooled and volume of erlenmeyerwas added water until reaching total 100 mL of that erlenmeyer. Mixture was taken 5 ml and poured into other erlenmeyer. Acetic acid 1 N and iodine solution were added 1 ml and 2 ml, respectively. Volume of erlenmeyer was made up to 100 ml with distilled water, waited for 20 min and then absorbance was measured at 620 nm by spectrophotometer (Model UV2, Unicam, England) (Juliano, 1971).

2. Crude Fiber

Samples 2 g were put into flask; added 200 mL hot H_2SO_4, and then heated at 100^o C for 30 min. Residue was separated by filter, mixed with 200 mL NaOH 1.25% solution and heated-stirred again at 100^o C for 30 min. After cooling down, residue was separated and washed with hot water and ethanol 95%. Residue was dried, weighed, incinerated at 400^o C and reweighed. Crude fiber calculation:

$$\text{Crude fiber} = \frac{weig\,ht\ loss\ in\ furnace}{weig\,ht\ of\ sample} \text{x } 100 \qquad (1)$$

(AOAC, 2002).

3. Moisture

Sample 5 g was put into petri disk which known weight, then put into an oven pre-set at 110^o C for 3 h. Sample was cooled in desiccators and reweighted, then returned into oven at 110^o C for 30 minutes until constant weight was obtained (AOAC, 2004).

$$\text{Moisture content} = \frac{weig\,ht\ of\ initial\ \ sample - weig\,ht\ of\ \ final\ \ sample}{weig\,ht\ of\ \ initial\ \ sample} \text{x } 100 \qquad (2)$$

4. Protein

Crude protein was determined by Kjeldahl method. Sample 0.5 g was put in digestion tube. Concentrated H_2SO_4 and catalyst ($CuSO_4$: K_2SO_4, 0.5: 1 w/w) was added 10 mL and 1 g, respectively, thendisgested in a digester at 420^o C for 1 h to

liberated nitrogen bond and form ammonium sulphate. Destilled water and NaOH 40% were added 10 ml and 85 ml respectively into the tube. The distillate 25 mL was gotten, added 4% boric acid and indicator (mixing of 0.1% (w/v) of bromocresol green and 0.1% (w/v) of methyl red. Titration usedHCl 0.1 until color changes (AOAC 2002).

$$\text{Total Nitrogen} = \frac{titration\ \ volume\ \ x\ N\ Hcl\ x\ 14.007}{weig\,ht\ of\ \ sample} \text{x } 100 \qquad (3)$$

$$\text{Protein content} = \%\ \text{total N x } 6.25 \qquad (4)$$

5. Fat

Crude fat of 2 g sample was determined by AOAC method using Soxtec system (Model HT6, Tecator, Sweden). Crude fat was extracted from sample with 60 mL Petroleum ether which put in weighted glass cup and evaporated 110^{o} C for 30 min for immersion, 30 min for washing and 60 min for recovery time. Yield was dried at 100^{o}C, cooled down and weighed.

$$\text{Crude fat} = \frac{Weig\,ht\ of\ \ cup\ \ after\ \ extraction\ \ -initial\ \ weig\,ht\ of\ \ cup}{weig\,ht\ of\ \ sample} \text{x } 100 \qquad (5)$$

6. Ash

Sample (5 g) was incinerated at 600^{o} C for 3 h in muffle furnace (Model FSE 621-210D, Sanyo Gallenkamp, UK). Previously, silica dish was weighted. After incinerating process, the disk and sample was cooled in desiccator and weighed again.

$$\text{Ash content} = \frac{Weig\,ht\ of\ \ residues\ \ after\ \ incineration}{weig\,ht\ of\ \ sample} \text{x } 100 \qquad (6)$$

7. Carbohydrate

Carbohydrate content was measured from the total (100) minus of contents of protein, fat, ash, and fiber

Carbohydrate = 100 – (crude fiber + protein + fat + ash) (7)

8. Pasting Properties

Peak properties were measured by Rapid ViscoAnalyzer (Model 4, Newport Scientific Pvt., Ltd. Australia). Sample (2.5 g) were kept into canister and mixed with 25 ml distilled water. Suspended sample was kept at 50^{o} C for 1 minute, then temperature was increased until reached 95^{o}C, kept for 3.2 min, and then decreased to 50^{o} C. Sample was mixed and homogenized with 960 rpm for 10 seconds during starting of test, then decrease 160 rpm and continued it throughout.

9. Swelling Power and Solubility

Swelling power and solubility were analyzed according to Konik's et al (1993) method. One gram of sample was dispersed in 50 ml distilled water in centrifuge tubes, then heated into water bath at different temperatures (60-95^{o} C) for 30 min with continuous stirring. Sample was cooled, centrifuged at 3000 rpm for 15 min. Supernatant was dried at 105^{o} C for 5 h. solubility of that sample can be calculated. While, for swelling determination, wet sample (sediments) was weighted.

$$\text{Solubility (\%)} = \frac{Weight\ of\ dry\ sample\ \ in\ supernatant}{weight\ of\ dry\ sample} \text{x } 100 \quad (8)$$

$$\text{Swelling power (\%)} = \frac{Weight\ of\ wet\ residue}{weight\ of\ dry\ sample} \text{x } 100 \quad (9)$$

10. Water Holding Capasity (WHC)

Sample (1 g) was dispersed in 50 ml distilled water into centrifuge tube, heated into water bath at different temperature (40-90^{o} C) for 30 min with continuous stirring. Treated sample was cooled at room temperature, and then centrifuged at 3000 rpm for 20 min. Separated supernatant, sediment was weighted and dried.

$$\text{WHC} = \frac{Weig\,ht\ of\ wet\ residue - weig\,ht\ of\ dry\ residue}{weig\,ht\ of\ dry\ residue} \text{x } 100 \qquad (10)$$

11. UV visible spectrometer analysis

Sample (1 g) was dispersed in 50 ml distilled water then heated in water bath at 95^{o}C for 30 min with continuous shaking and then cooled at 25^{o}C. Gelatinized starch (10 ml) was put in Erlenmeyer, added distilled water 25 ml and neutralized with HCl 0.1 M until pH 3. The suspension was mixed with 100 ml distilled water and 0.5 ml of iodine solution. The absorbance was measured at 190-900 nm by UV spectrophotometer ((Model UV2, Unicam, England) (Xin et al., 2012).

12. Scanning Electron Microscopy

The sample particle is sprayed onto the surface of metal plate covered with double-side tape, put into a vacuum chamber. The sample is observed in a tool-coated SEM (S-3400N HITACHI) with an accelerating voltage of 20 kV (Maulani R.R et al., 2013).

III. For emulsion samples

1. Emulsion capacity and emulsion stability

Emulsion capacity and stability was estimated according to Ahmedna et al., (1999) and Abdul et al.,(2000) with some modification. Sample gotten in each emulsion was centrifuged at 2,100xg for 30 min. The ratio of the height of the emulsified phase to the height of total liquid was emulsion capacity (%) After that, the homogenized sample was incubated at 45^{o}C for 30 min and centrifuged at 2,100xg for 30 min. The ratio of the height of the emulsified phase to the height of total liquid was emulsion stability (%).

2. Peroxide value

Sample (2 ml) was dissolved with 20 ml acetic acid- chloroform (3:2) solution. 0.25 ml of KI 95% was added, incubated shaking water bath 25^{o}C for 1 min and added 12 ml distilled water. Sample was titrated with 0.01 N sodium thiosulphate solutions until the color changing (transparent). Indicator of soluble starch 1 % was used.

Peroxide value (meq/kg sample) = (Sx M x 1000)/ ml of sample (11)

Where: S = ml of sodium thiosulphate
M= 0.01, concentration of sodium thiosulphate

(AOAC, 1990).

3. Anisidine value

Sample (0.5 ml) was put in volumetric flask 25 ml and made up to volume with isooctane. The absorbance (Ab) of the resulting solutions at 350 nm was determinate. Besides that 5 ml of each solution was pipette into a test tube and reagent also, and then para-anisidine solution (para-anisidine dissolved in acetic acid 0.25 g/100 ml solution) was added to each tube and mixed well. After 10 min, the absorbance (As) of the sample solutions was read. Anisidine value was calculated:

Anisidine value = [25x (1.2 As-Ab)]/ ml of sample (12)

4. Color Spectra

Color spectra were measured by a Hunter Lab Spectrocolorimeter (Model TC-P III A, Tokyo Denshoku Co., Ltd., Japan). Samples (10 ml) were put into specificcontainer of the instrument, closed the instrument and measured spectra of each sample. Lab system was used, where L* (L*= null means black and L*= 100 means white), a* (-a*= greenness and +a*= redness) and b* (-b*= blueness and +b= yellowness).

5. Viscosity

Sample were determined at 25^{o}C using Brookfield viscometer (model No-LV DV-II+PX, USA), transferred to a 100 ml beaker and leveled up to the brim. The spindle number 61 was used for all emulsions at the speed of 5 rpm.

3.3 Statistical Analysis

All experiment will be done in triplicate, and means ± in standard deviations.Analysis of variance used ANOVA procedures.

CHAPTER 4
RESULTS AND DISCUSSION

4.1 Native Sago Starch Analysis

Native sago starch was analysed for amylose and amylopectin contents.In present study,amylose content of native sago starch was 41.14% whereas its amylopectin content was 58.86%. Anggraini et al., (2013) also found that amylose and amylopectin content of native sago starch were 41% and 59% respectively. Amylose and amylopectin content of starch will affect on resistant starch (RS) formation. Thus, before producing of resistant, it should be known amylose content of raw material.In this research, native sago starch used is proper to product resistant starch type 3 (RS_3). Aparico et al., (2005) found amylose content of banana starch was 37% and it can produce RS_3 around 45.5%. Nasrin et al., (2014) also reported that amylose content of culled banana starch was 39.8% and it can produce RS around 13%.

4.2 Resistant Starch Contents

Resistant starch (RS) contents of lintnerized starch and lintnerized-autoclaved starch by variation of time and citric acid concentration are shown in Table 4.1. In the present study, time variations of hydrolysis did not affect on the amount of RS content whereas RS value was affected by concentrations of citric acid. Highest RS contents were obtained of lintnerized starch by citric acid concentration of 2N. Previous studies reported that increased concentration of hydrochloric acid and followed by autoclaving-cooling treatment affected RS value (Zhao et al., 2009; Nasrin et al., 2014). Used citrit acid in this study reacted with sago starch, resulting chemically modified starch (esterified starch) which strengthened starch structure, thus increasing RS yield. In contrast, RS yields were decrease of variation of time when sago starch was only hydrolyzed by citric acid without

autoclaving-cooling treatment. Partial acid hydrolysis broke down the amylopectin structure, generating short linear chains so that increasing the mobility of molecules. When autoclaving-cooling treatment, these chains rearranged and recrystallized, forming resistant product which have tightly packed structure stabilized by hydrogen bonding while only partial acid hydrolysis was treated, the resistant product cannot be formed. Therefore lintnerized starch generated low RS.

Table 4.1 RS contents of lintnerized starch and lintnerized-autoclaved starch

Time of hydrolysis (h)	Concentration of Acid (N)	RS value (%)	
		Lintnerized-autoclaved	Lintnerized
3	1	35.49 ± 0.003	1.24 ± 0.001
	1.5	40.32 ± 0.002	1.24 ± 0.003
	2	40.32 ± 0.002	1.54 ± 0.001
6	1	34.71 ± 0.001	1.24 ± 0.003
	1.5	34.71 ± 0.003	0.96 ± 0.004
	2	40.32 ± 0.001	1.55 ± 0.001
12	1	35.49 ± 0.001	1.10 ± 0.002
	1.5	38.68 ± 0.004	0.72 ± 0.002
	2	40.32 ± 0.001	1.10 ± 0.004

Data were mean and standard deviations of three determinations.

4.3 Chemical composition

Chemical compositions (amylose, crude fiber, moisture, protein, fat, ash and carbohydrate) both highest RS value of lintnerized starch and lintnerized-autoclaved starch were compared by composition of native sago starch and hydrolyzed starch by distilled water (Table 4.2). Amylose content of native starch obtained 41.14% was higher than Ahmad's report (1999) around 24-31%. Amylose of lintnerized-autoclaved starch was highest than other samples. It was indicated that sample had compact structure compared others. Hydrolyzed starch by distilled water followed autoclaving without cooling resulted lowest amylose

content. Ozturk et al., (2011) reported that after autoclaving, RS value was decrease. Indeed, autoclaving-cooling treatments gave big effect on RS value. Zhao et al., (2009) found effect of cycle times of autoclaving-cooling of maize starch on RS.Maize starch was dispersed in distilled water then autoclaved-cooled, repeated 1-5 times, increasing RS value. Moreover, this case was proved, when hydrolyzed starch by citric acid without autoclaving-cooling treatment resulted low RS and low amylose content.Sandhu, Singh and Lim (2007) reported decreased of amylose contents (from 16.9 % to 13.3 %) after hydrolyzed of corn starch by acid. Atichokudomchai et al., (2000) explained the decreased of amylose content during acid hydrolysis acid that acid attacked the amorphous regions mostly where amylose resides.

Native sago starch had thehighest protein and fat than others. Hydrolysis by citric acid decreased significantly protein and fat contents. Also after autoclaving-cooling treatment, protein and lipid content decreased, because heat treatment can denature protein and saponify fat which became soluble. Crude fiber content of lintnerized-autoclaved starch was highest than native and lintnerizedstach. It was related with re-associate the structure when gelatinization and retrogradation process. Moisture and ash of native sago showed the nearly result with Ahmad's result (1999). Moisture content of most native starches was around 12% at ambient temperature and humidity conditions.Lintnerized-autoclaved starch had the lowest moisture (8.33 ± 0.1). This case can be also correlated with its compact and rigid structure than other samples (see 4.4 microstructure analysis).

Table 4.2 Chemical compositions of native sago starch, hydrolyzed starch by water (DW), lintnerized starch (L) and lintnerized-autoclaved starch (LA)

Chemical composition	Amount of content (%)			
	Native	DW	L	LA
Amylose	41.14 ± 0.006	30.14 ± 0.001	36.52 ± 0.001	57.20 ± 0.006
Amylopectin	58.86 ± 0.006	69.86 ± 0.001	63.48± 0.001	42.8 ± 0.006
Carbohydrate	97.33 ± 0.017	95.22 ± 0.001	97.31 ± 0.006	96.22 ± 0.025
Protein	0.58 ± 0.058	0.35 ± 0.001	0.26 ± 0.001	0.15 ± 0.058
Fat	1.67 ± 0.006	1.0 ± 0.000	0.83 ± 0.006	0.50 ± 0.000
Ash	0.36 ± 0.000	1.44 ± 0.001	0.45 ± 0.002	0.32 ± 0.001
Crude fiber	0.06 ± 0.005	1.99 ± 0.035	1.15 ± 0.015	2.5 ± 0.044

1. Data were mean and standard deviation of three determinations.
2. Dry basis
3. Production of lintnerization starch uses citric acid 2 N for 12 h.
4. Production of lintnerization-autoclaved starch uses citric acid 2 N for 12 h, and it is autoclaved at 135^{o}C for 30 min and cooled 4^{o}C. Autoclaving-cooling treatments were repeated three times at same temperature and time.

4.4 Microstructure analysis

Scanning electron micrographs of native sago starch, hydrolyzed starch by distilled water, lintnerized and lintnerized-autoclaved starch were presented in Figure 4.1 - Figure 4.4. The native starch granules were found to be oval to round shaped with well defined edges compared other samples. From that figure also looked that native starch has the smallest granules. However, as shown in figure 4.2 and 4.3 the starch granules lost their smoothness and structural integrity. Moreover starch granules of lintnerized starch are largely amorphous structure. Starch granules of lintnerized-autoclaved were dense and rigid structure, indicating it formed crystalline structure after gelatinization and retrogradation process.

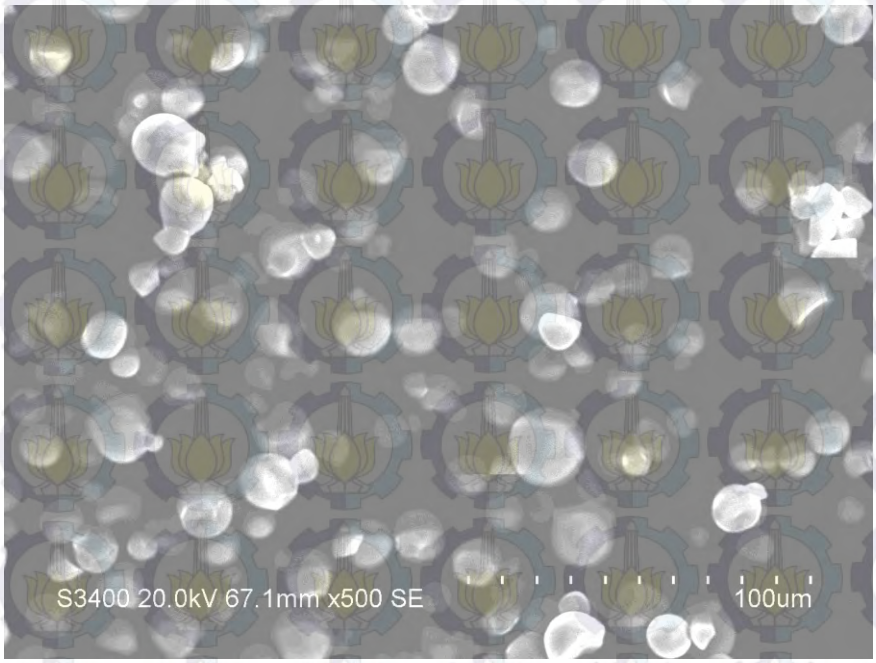

Figure 4.1 Granule morphology of sago starch

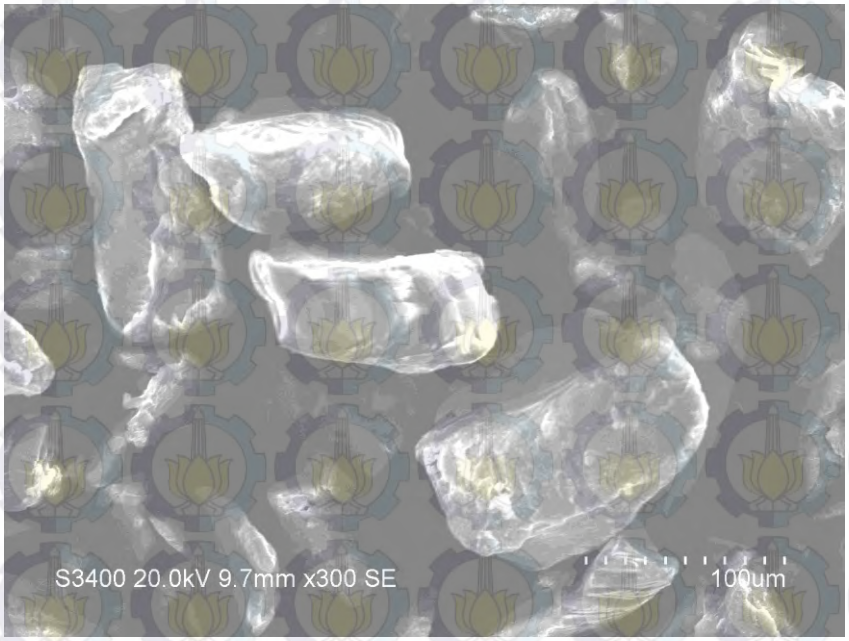

Figure 4.2 Granule morphologyof hydrolyzed starch by distilled water

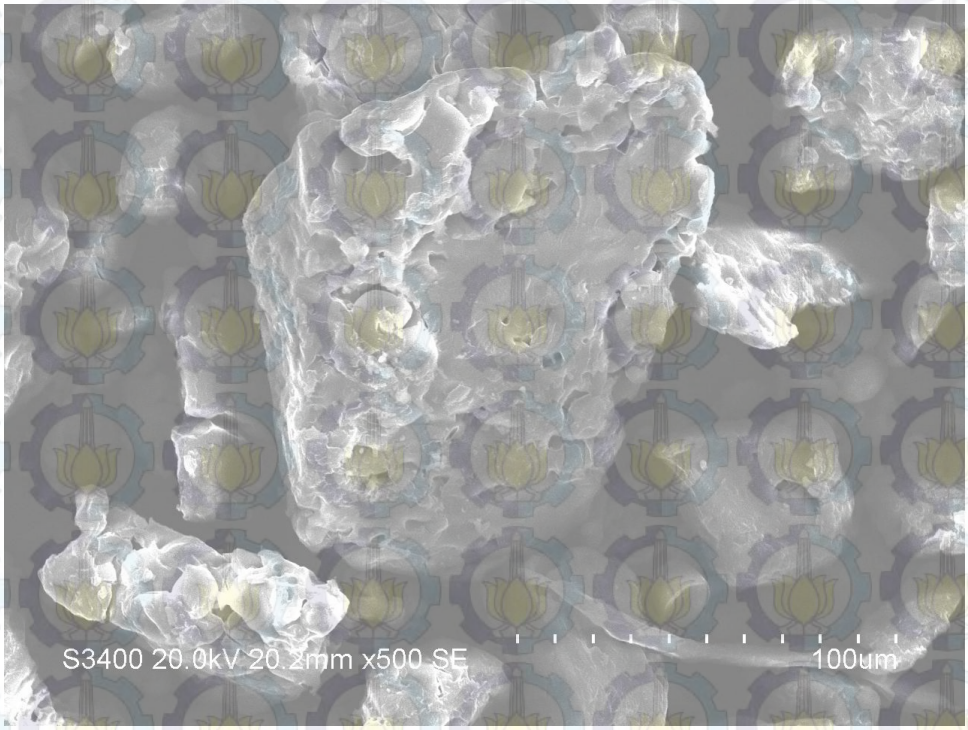

Figure 4.3 Granule morphologyof lintnerized starch

Figure 4.4 Granule morphologyof lintnerized-autoclaved starch

4.5 UV/visible spectra analysis

UV/visible spectra exhibited the amylose-iodine complex at 550-600 nm. From the figure 4.5 there were the difference absorbances of native starch, hydrolyzed starch by distilled water, lintnerized starch and lintnerized-autoclaved starch. The highest intensity of peak was reached by lintnerized-autoclaved starch whereas the lowest intensity of peak was reached by lintnerized starch. Nasrin and Anal (2014) also reported intensity of peak from lintnerized starch of culled banana pulp starch as the lowest value. The present study, values of intensity of peak were lower than lintnerized-autoclaved of culled banana pulp starch, the absorbance was around 0.9.

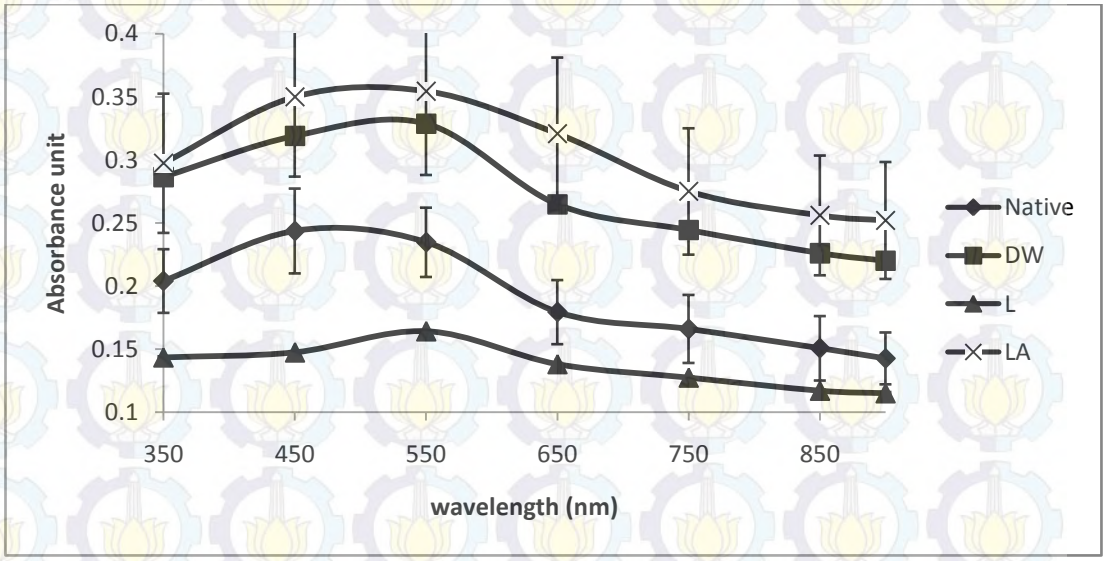

Figure 4.5UV/visible spectra of native sago starch, hydrolyzed starch by distilled water (DW), lintnerized starch (L) and lintnerized-autoclaved starch (LA)

4.6 Pasting Properties

The pasting properties of native sago starch, hydrolyzed starch by distilled water, lintnerized starch, and lintnerized-autoclaved starch were analyzed by RVA (Table 4.3). All of the viscosity values (except peak time and pasting temperature) of modified starch samples were found to be less than those of the native starch. Pasting temperature (^{o}C) of hydrolyzed starch by distilled water was higher than native starch while pasting temperature of lintnerized starch and lintnerized-autoclaved starch were not detected. These values are similar with Nasrin's report (2014), when lintnerized starch using 1 N hydrochloric acid still showed 87.5^{o}C of pasting temperature but lintnerized starch used 1.5 N and 2 N hydrochloric acid, the pasting temperatures (^{o}C) were not detected. Acid hydrolysis caused reduction in the molecular weight of starch, thus the viscosity decreased significantly (Wang L., 2001).Setback value of lintnerized-autoclaved starch was lowest than other samples, indicating that sample had highest retrogradation while through viscosity represented lowest viscosity measuring the capacity of paste to hold out breakdown during cooling.

Table 4.3 Pasting properties of native sago starch, hydrolyzed starch by distilled water, lintnerized starch and lintnerized-autoclaved starch.

Properties	Sample			
	Native	DW	L	LA
Peak viscosity (RVU)	403.03 ± 34.95	75.00 ± 7.32	23.33 ± 5.46	15.25 ± 3.44
Through (RVU)	146.17 ± 5.48	42.89 ± 1.69	22.17 ± 5.08	11.19 ± 1.48
Break down viscosity (RVU)	256.86 ± 35.44	32.11 ± 5.71	1.17 ± 0.38	4.05 ± 4.79
Final viscosity (RVU)	199.72 ± 8.07	50.28 ± 3.39	29.39 ± 5.92	13.22 ± 1.72
Setback viscosity (RVU)	53.56 ± 5.58	7.39 ± 1.92	7.22 ± 1.71	2.03 ± 0.43
Peak time (min)	3.49 ± 0.14	4.62 ± 0.17	6.71 ± 0.08	4.46 ± 2.91
Pasting temperature (^{o}C)	50.57 ± 0.39	68.92 ± 3.08	ND	ND

Data were mean and standard deviation of three determinations.

Native = sago starch; DW = hydrolyzed starch by distilled water; L= lintnerized starch; LA = lintnerized-autoclaved starch

4.7 Solubility

Figure 4.6 shows thesolubility of native starch, hydrolyzed starch by distilled water, lintnerized starch and lintnerized-autoclaved starch. Solubility of lintenrized starch drastically increased than other samples. Nasrin et al., (2014) also found increased solubility of lintnerized starch using hydrochloric acid 2N compared lintnerized starch using hydrochloric acid 1N and 1.5 N. In this study, solubility at 95^{o}C of lintnerized-autoclaved sample (52.67%) was almost similar with native starch (52.00%), this value was difference with Nasrin's reported (2014) and Aparicio's reported (2005) that lintnerized-autoclaved sample from banana starch had the lower solubility than native starch. In contrast, Ozturk et al., (2011) reported that solubility of lintnerized-autoclaved from corn starch, Hylon V and Hylon VII samples obtained higher than native and linterized starch. It can be related with other effect such as lipid-amylose complex, protein-amylose complex. In this study, lipid and protein contents of native starch were highest than other samples,it may be due to the fact that there is formation of lipid and protein complexes with amylose so that decreasing the solubility value subsequently giving almost similar value with lintnerized- autoclaved starch.

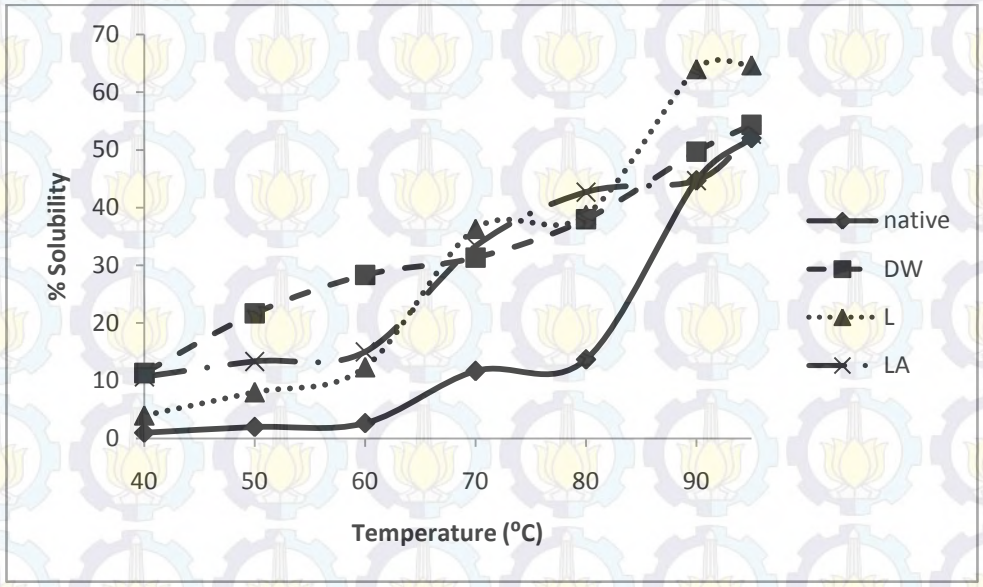

Figure 4.6 Solubility of native sago starch, hydrolyzed starch by distilled water (DW), lintnerized starch (L) and lintnerized-autoclaved starch (LA).

4.8 Swelling power

Swelling power indicated the extent of interaction within amorphous and crystalline areas of starch granules. Swelling power also related with solubility value, influenced not only protein-amylose complex and lipid-amylose complex but also amylose-amylopectin ratio, degree of branching, length of branches, configuration of the molecules (Ratnayake et al., 2002 and Han et al., 2002). During swelling process, starch granules obtained thermal energy which looses the intra granular bonds and thengranules absorbed water. The starch granule which had low molecular weight of amylose will soluble easily and released out of the granules into surrounding medium. By shaking way during process, it can faster break down internal granular bonds so that it caused enormous swelling (Nasrin et al., 2014).

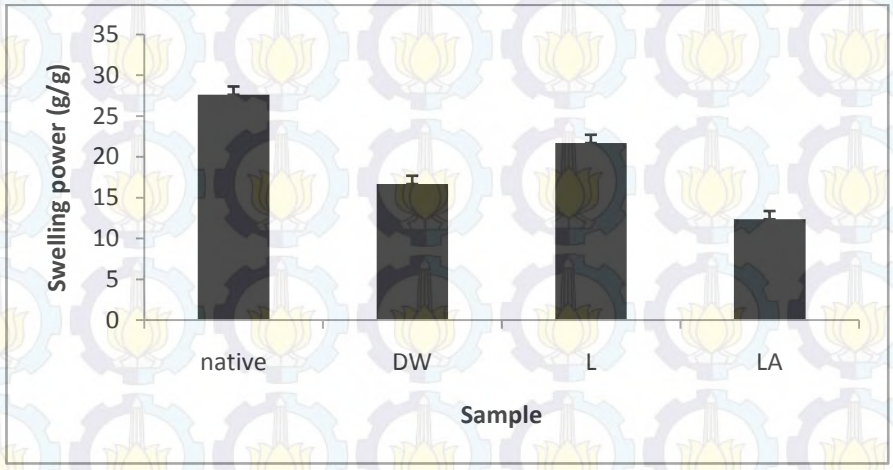

Figure 4.7 Swelling power of native sago starch, hydrolyzed starch by distilled water (DW), lintnerized starch (L) and lintnerized-autoclaved starch (LA)

From Figure 4.7, the lowest value of swelling power at 95^{o}C was lintnerized-autoclaved starch (12. 37%) whereas the highest value at the same temperature was native starch (27.62%). Swelling power of lintnerized starch was 21.71% and for hydrolyzed starch by distilled water was reached 16.69%. In theoretically,lintnerized-autoclaved samples presented lower swelling power (lower water retention features) than native and lintnerized starch. Aparicio et al., (2005) and Nasrin et al., (2014) also found that swelling power of lintnerized-autoclaved starch was lower than native and lintnerized starches.

4.9 Water holding capacity

Water holding capacity (WHC) expressed the ability of sample to retain its inherent moisture even though heating was applied to it. Rodriguez et al., (2008) explained that WHC was largely influenced by physical conditions of starch granules, dietary fibre, protein, and amylose contents in the sample. The WHC value at different temperature (40^{o}C-90^{o}C) of native sago starch, hydrolyzedstarch

by distilled water, lintnerized starch and lintnerized-autoclaved starch were exhibited in Figure 4.8. The lowest value of WHC at 90°C was lintnerized-autoclaved starch (10.52%) whereas the highest value of WHC was native starch (20.30%), which proportional with swelling power value. Hydrolyzed starch by distilled water also can hold water capacity (11.79%) compared lintnerized starch (18.39%). Lintnerized starch had most number of available binding sites for water as containingamorphous region in the starch granule. Thus, it made easily to absorb excess water. Lintnerized-autoclaved starch which had dense and compact structure can retain its inherent moisture, resulting lowest of WHC value.

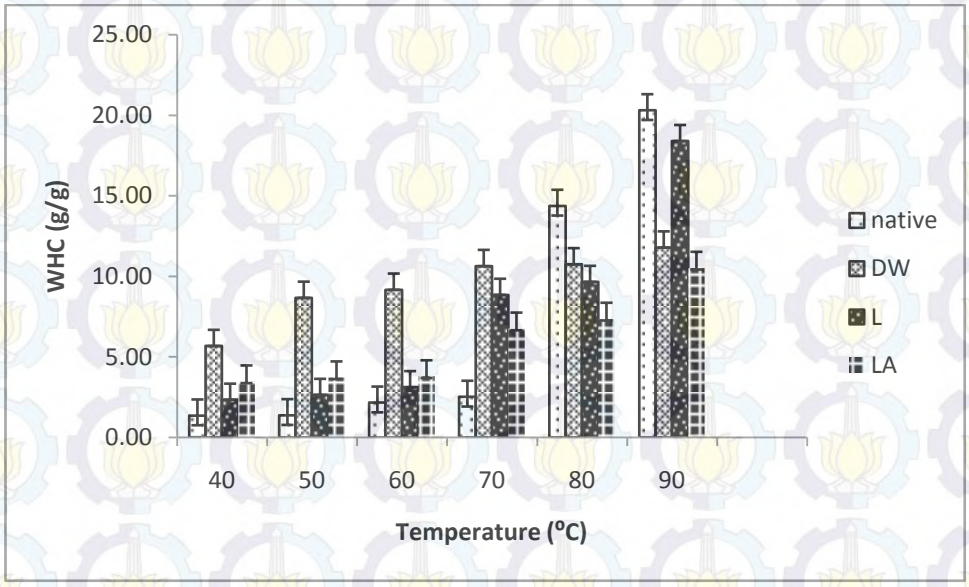

Figure 4.8 Water holding capacity of native sago starch, hydrolyzed starch by distilled water (DW), lintnerized starch (L) and lintnerized-autoclaved starch(LA)

4.10 Production Fish oil Emulsions from RS and Casein compared Emulsion produced using RS and Soy Protein Isolate (SPI)

1. Viscosity and color values of emulsions

Viscosities of fish oil emulsions made from RS-casein and RS-SPI were showed in Table 4.4. The ranges of emulsions type from RS-Casein were lower (20.00cP-31.99 cP) than those of RS-SPI (37.05cP-52.07 cP). Nasrin et al., (2014) reported that ranges of viscosity of fish oil emulsions made from mixture of culled banana pulp resistant starch and SPI were (34.60-146.48 cP). For comparison, emulsions from emulsifier (SPI or casein) and Hylon VII were made, resulting lower viscosity compared emulsions made from emulsifier and RS. In each type of emulsions obtained that viscosity increased with decreasing oil load.

Color values of each emulsion were also showed in Table 4.4. The highest L* value of RS-casein emulsions was 84.40, made from 5% casein+5% Hylon VII+ 5% fish oil and the lowest value was 74.33, made from 3.75% SPI+ 3.75% RS+7.5% fish oil, while highest L* value of RS-SPI emulsion was 85.34, made from 7.5% SPI and 7.5% fish oil, and lowest value was 82.48, made from 5% SPI+ 5% RS+5% fish oil. In this research, the lowest L* value was obtained from mixture RS and emulsifiers (casein or SPI). In contrast with Nasrin's report that lowest of L* value was made from only mixture of SPI and oil without resistant starch. The L* value decreased in emulsions made from RS-SPI, similarly with Nasrin's report. All emulsions had –a* value (greenness), conversely this value also was different with Nasrin's report that emulsion made from only SPI had +a* (redness), but when using mixture of SPI and culled banana pulp resistant starch showed the same color (greenness). The highest b* value of RS-casein emulsions was gotten 5.53 (7.5% casein+ 7.5% fish oil) and the lowest b* value was 2.17 (10% casein + 5% fish oil), whereas the highest b* value of RS-SPI emulsions was gotten 14.68 (5% SPI + 5%RS + 5% fish oil) and the lowest b* value was

12.39 (3.75% SPI + 3.75% Hylon VII + 7.5% fish oil). Decreasing b* value of RS-SPI emulsions was proportional with reducing oil load.

Table 4.4 Viscosity and color value of fish oil emulsion from RS-Casein and RS-SPI

Source of fish oil Emulsion	Emulsion type	Viscosity (cP)	Color value		
			L*	a*	b*
RS and Casein	E_1	31.99 ± 0.69	82.14 ± 0.18	-2.81 ± 0.05	5.53 ± 0.28
	E_2	49.19 ± 0.00	74.33 ± 0.09	-2.02 ± 0.06	2.63 ± 0.14
	E_3	30.37 ± 0.65	79.33 ± 0.13	-2.06 ± 0.06	2.99 ± 0.19
	E_4	30.79 ± 0.69	78.09 ± 0.18	-1.97 ± 0.08	2.17 ± 0.36
	E_5	38.52 ± 0.12	78.63 ± 0.11	-1.98 ± 0.06	3.00 ± 0.21
	E_6	20.00 ± 0.69	84.40 ± 0.15	-2.21 ± 0.10	4.02 ± 0.24
RS and SPI	E_1	52.07 ± 1.35	85.34 ± 0.10	-2.91 ± 0.09	13.39 ± 0.19
	E_2	46.22 ± 0.51	84.80 ± 0.15	-2.12 ± 0.07	13.99 ± 0.38
	E_3	41.50 ± 0.55	85.32 ± 0.24	-2.71 ± 0.07	12.39 ± 0.41
	E_4	43.64 ± 0.46	83.5 ± 0.14	-2.33 ± 0.07	14.59 ± 0.29
	E_5	43.27 ± 1.78	82.48 ± 0.27	-2.25 ± 0.04	14.68 ± 0.48
	E_6	37.05 ± 0.68	83.78 ± 0.20	-2.5 ± 0.05	12.93 ± 0.39

Data were mean and standard deviation of three determinations.

E_1= 7.5% emulsifier (casein or SPI) + 7.5% fish oil; E_2= 3.75% emulsifier + 3.75% RS + 7.5% fish oil; E_3= 3.75% emulsifier + 3.75% Hylon VII + 7.5% fish oil; E_4= 10% emulsifier + 5% fish oil; E_5= 5% SPI + 5% RS + 5% fish oil; E_6= 5% Hylon VII + 5% fish oil.

2. Emulsion capacity and emulsion stability values

Emulsion capacities of fish oil emulsion made from RS-casein and RS-SPI were showed in Figure 4.9. The highest of emulsion capacity made from RS-casein was obtained 5.67 % (3.75% casein+ 3.75 RS + 7.5% fish oil) while the highest that of RS-SPI was obtained 11.33% (5% SPI + 5% RS + 5% fish oil). In the present study, when using 3.75% SPI+ 3.75 RS + 7.5% fish oil, the result also gave almost similar (11.00%). Even using 5% casein + 5% RS + 5% fish oil, the valueof emulsion capacity gave almost similar (5.33%), compared using 3.75%

casein+ 3.75 RS + 7.5% fish oil. Emulsion capacity made from only emulsifier (casein or SPI) with fish oil showed the lower value. When compared emulsion from Hylon VII and emulsifier (casein or SPI), the emulsion capacity also showed lower value. These results indicated that RS may improve emulsifying characteristics. Ozturk et al., (2009) reported emulsion capacity value of mixture Hylon VII and albumin was gotten 12%, this result was higher than using mixture of Hylon VII and casein (3.33%), because the Ozturk's research didn't use the same amount of water in the emulsion system, thus the value of emulsion capacity was higher that this research.

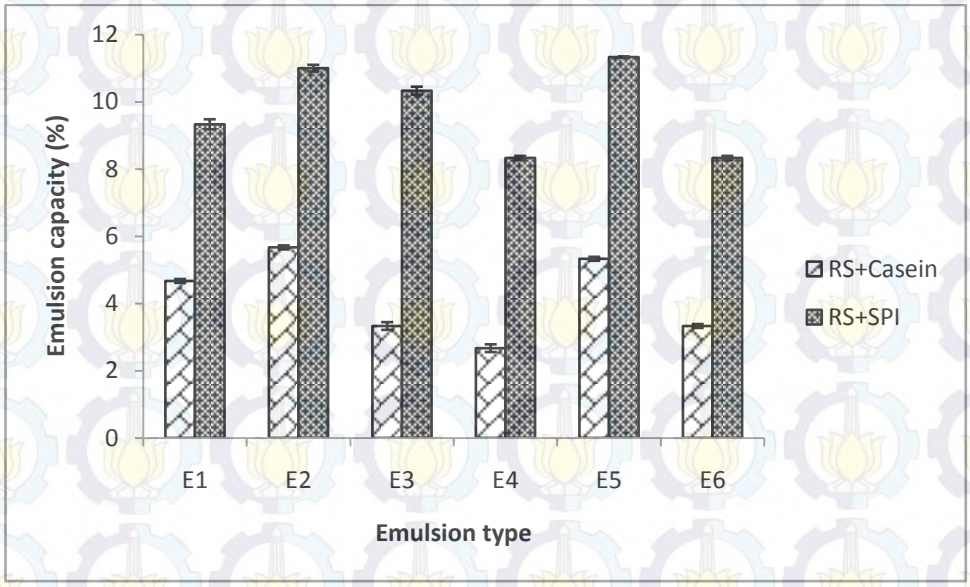

Figure 4.9 Emulsion capacity of RS and Casein compared Emulsion produced using RS and Soy Protein Isolate.

E_1= 7.5% emulsifier (casein or SPI) + 7.5% fish oil; E_2= 3.75% emulsifier + 3.75% RS + 7.5% fish oil; E_3= 3.75% emulsifier + 3.75% Hylon VII + 7.5% fish oil; E_4= 10% emulsifier + 5% fish oil; E_5= 5% SPI + 5% RS + 5% fish oil; E_6= 5% Hylon VII + 5% fish oil.

Emulsion stabilities (Figure 4.10) also exhibited that the highest value was gotten from mixture of emulsifier (Casein or SPI) and RS, but the higher value of emulsion capacity was obtained when using mixture of RS and SPI (11.33%) than that of RS and casein (8.00%).However, emulsion made from RS-casein showed increasing of fish oil load increased emulsion stability value. Ibrahim et al., (2012) and San et al., (2009) reported that the emulsion containing 10% oil was more stable than containing 5% oil, because at higher oil concentration, the packing fraction of oil droplets will increase so that enhancing viscosity of emulsion by reducing the creaming rate. Sun and Gunasekaran (2009) also found that the oil concentration played important role in determining emulsion stability.

On the other hand, all emulsions were immediately kept at cold temperature (4^oC) and room temperature (25^oC) after preparation of emulsions. Figure 4.11- 4.14 showed stability of fish oil emulsions stored at cold temperature and room temperature during storage period. All emulsions were damaged after 3^{rd} days kept in room temperature and all emulsions looked like stable in cold temperature at up 9^{th} days, only a little creaming for emulsion (E1) made from RS-casein, but it can mix well after shaking by hand (see circle mark in Figure 4.14).Mozyraityle et al., (2006) and Rahmani et al., (1998) reported that high temperature contributed to oxidize lipid rapidly and it will be two times more severe per 10^o rise in temperature, indicating that high temperature will break down the emulsions, make the emulsions were coalescence. In this study, all emulsions cannot keep in room temperature; they should be kept in cold temperature.

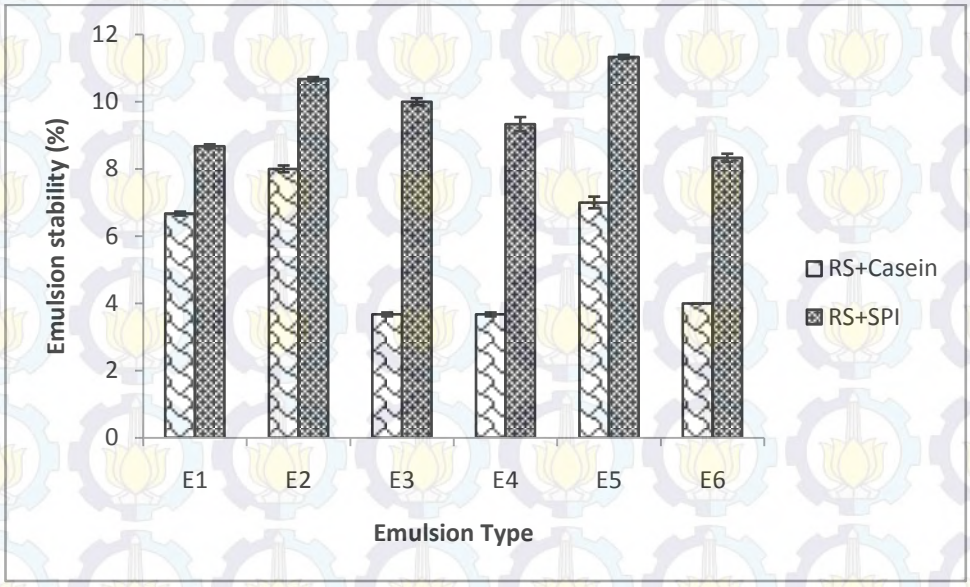

Figure 4.10 Emulsion stabilityof RS and Casein compared Emulsion produced using RS and Soy Protein Isolate

E_1= 7.5% emulsifier (casein or SPI) + 7.5% fish oil; E_2= 3.75% emulsifier + 3.75% RS + 7.5% fish oil; E_3= 3.75% emulsifier + 3.75% Hylon VII + 7.5% fish oil; E_4= 10% emulsifier + 5% fish oil; E_5= 5% SPI + 5% RS + 5% fish oil; E_6= 5% Hylon VII + 5% fish oil.

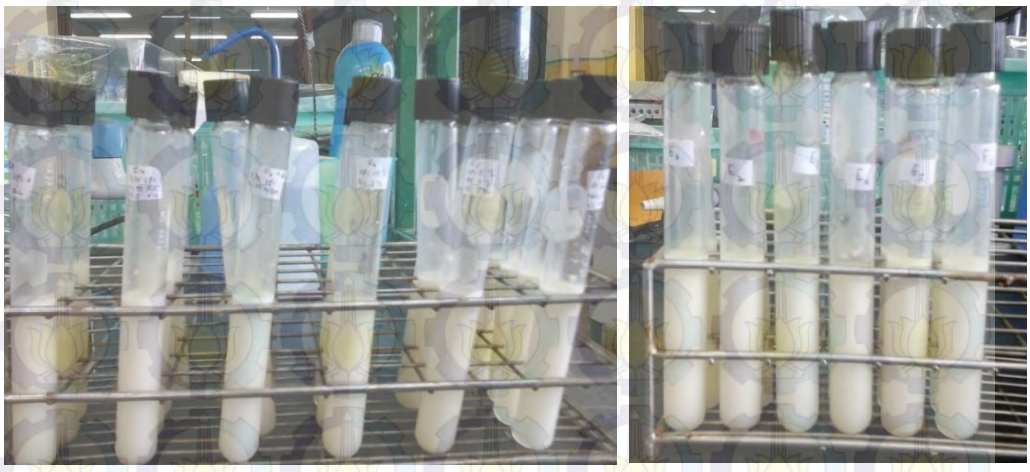

Figure 4.11 Stability of fish oil emulsions stored at 4^{o}C (left side) and 25^{o}C (right side) at 0th days of storage period.

E_1= 7.5% SPI + 7.5% fish oil; E_2= 3.75% SPI + 3.75% RS + 7.5% fish oil; E_3= 3.75% SPI + 3.75% Hylon VII + 7.5% fish oil; E_4= 10% SPI + 5% fish oil; E_5= 5% SPI + 5% RS + 5% fish oil; E_6= 5% Hylon VII + 5% fish oil.

Figure 4.12 Stability of fish oil emulsions stored at 4°C (left side) and 25°C (right side) at 0th days of storage period.

E_1= 7.5% casein + 7.5% fish oil; E_2= 3.75% casein + 3.75% RS + 7.5% fish oil; E_3= 3.75% casein + 3.75% Hylon VII + 7.5% fish oil; E_4= 10% casein + 5% fish oil; E_5= 5% casein + 5% RS + 5% fish oil; E_6= 5% Hylon VII + 5% fish oil.

Figure 4.13 Stability of fish oil emulsions stored at 4°C (left side) and 25°C (right side) at3rd days of storage period.

E_1= 7.5% SPI + 7.5% fish oil; E_2= 3.75% SPI + 3.75% RS + 7.5% fish oil; E_3= 3.75% SPI + 3.75% Hylon VII + 7.5% fish oil; E_4= 10% SPI + 5% fish oil; E_5= 5% SPI + 5% RS + 5% fish oil; E_6= 5% Hylon VII + 5% fish oil.

Figure 4.11 shows stability of fish emulsion at 0^{th} days made from RS and SPI where as Figure 4.12 shows emulsion made from RS and casein. At 0^{th} days all these emulsion looked like the same at room temperature and cold temperature, but after 3^{rd} days of storage periods, emulsions kept in room temperature were broken, figure 4.11 (right side) and figure 4.12 (right side) show differences in damages of emulsions made from RS-SPI and RS-casein. Emulsions made from RS-SPI occurred sedimentation, large droplets were moving faster to the bottom because the density was larger than that of the medium but emulsion (E_1) occurred flocculation, an aggregation of the droplets into larger units without any change in primary droplet size (Tadros, 2013). Besides that, emulsions made from RS-casein also underwent flocculation and a little sedimentation compared emulsions were made from RS-SPI. E_1 and E_3 from RS-casein emulsions looked like change the color to yellow; it may be influenced by casein which had also yellow color, because of instability condition, it changed the color of emulsion.

Figure 4.14 Stability of fish oil emulsions stored at 4^oC (left side) and 25^oC (right side) at 3^{rd} days of storage period.

E_1= 7.5% casein + 7.5% fish oil; E_2= 3.75% casein + 3.75% RS + 7.5% fish oil; E_3= 3.75% casein + 3.75% Hylon VII + 7.5% fish oil; E_4= 10% casein + 5% fish oil; E_5= 5% casein + 5% RS + 5% fish oil; E_6= 5% Hylon VII + 5% fish oil.

3. Peroxide and anisidine values of RS and Casein compared Emulsion produced using RS and Soy Protein Isolate

Peroxide value (PV) and anisidine value (AV) were a measure of oxidation or rancidity. PV is an indicator of initial stages of oxidative change, whereby a lipid can be decay or still stable of hydroperoxide concentration by monitoring the amount of hydroperoxides as a function of time. Hydroperoxide is called as primary oxidation products and unstable, so that being susceptible to decomposition become the secondary oxidation products such as aldehydes, ketones, alcohols, epoxy compounds. One of Methods for knowing secondary oxidation products was through anisidine value. AV method measures the content of aldehydes generated during the decomposition of hydroperoxide (Shahidi et al., 2002; Riuz. et al., 2001; Doleschall et al., 2002).

From Figure 4.15-4.18, PV and AV of each emulsion increased with increasing storage time. Peroxide values of emulsions made from RS-casein at the storage time were higher that these of emulsion made from RS-casein. Emulsion made from 5% SPI+ 5% RS+ 5% fish oil (E_2) had the lowest of peroxide value (1.67 meq/L) compared other emulsions (Figure 4.15 and 4.16) and also emulsion made from 5% casein+ 5% RS+ 5% fish oil (E_2) had the lowest of peroxide value (3.67 meq/L) if compared with emulsion made from only casein or mixture of casein and Hylon VII (Figure 4.15). At the 9^{th} days of storage period, PV of E_2 made from SPI+RS was 6.33 meq/Lwhere as PV of E_2 made from casein+RS was 6.67 meq/L. RS may contribute in this emulsion so that resulting the lowest PV. RS was high amount of crystallinity than HylonVII which was only as native starch, thus emulsion made from Hylon VII had higher access of oxygen to oxidize the

fish oil than emulsion made from RS. The highest of PV was gotten emulsion made from 10% casein+5% oil (25.00 meq/L) and also from 10% SPI+5% fish oil (24.33meq/L). In this present study, emulsifier (casein or SPI) gave high effect because emulsion made from 7.5% emulsifier (casein or SPI)+ 7.5% fish oil result PV < 10 meq/L. Nasrin et al., (2014) reported that emulsion made only 7.5% SPI + 7.5% fish oil were more susceptible to oxidation that made by 10% SPI + 5% oil.

Anisidine values of each emulsion were shown in Figure 4.17-4.18. Each emulsion made from RS-casein had lower value than made from RS-SPI. However, emulsion made from 5% emulsifier (casein or SPI)+ 5% RS+ 5% fish oil was lower value than other emulsion systems. At the 0^{th} day, the AV made from 5% SPI+ 5% Hylon VII+ 5% fish oil were the highest value (4.86) compared other emulsions (AV< 2), but that value was still lower than Nasrin's report which showed that AV of all emulsions at 0^{th} days were more than 6.

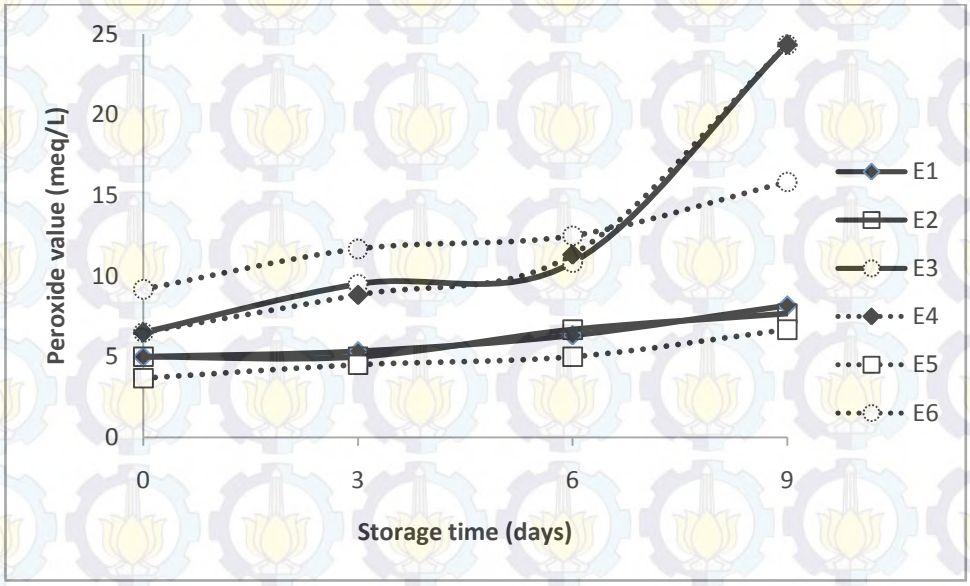

Figure 4.14 Peroxide valueof emulsions from RS and Casein

E_1= 7.5% casein + 7.5% fish oil; E_2= 3.75% casein + 3.75% RS + 7.5% fish oil; E_3= 3.75% casein + 3.75% Hylon VII + 7.5% fish oil; E_4= 10% casein + 5% fish oil; E_5= 5% casein + 5% RS + 5% fish oil; E_6= 5% Hylon VII + 5% fish oil.

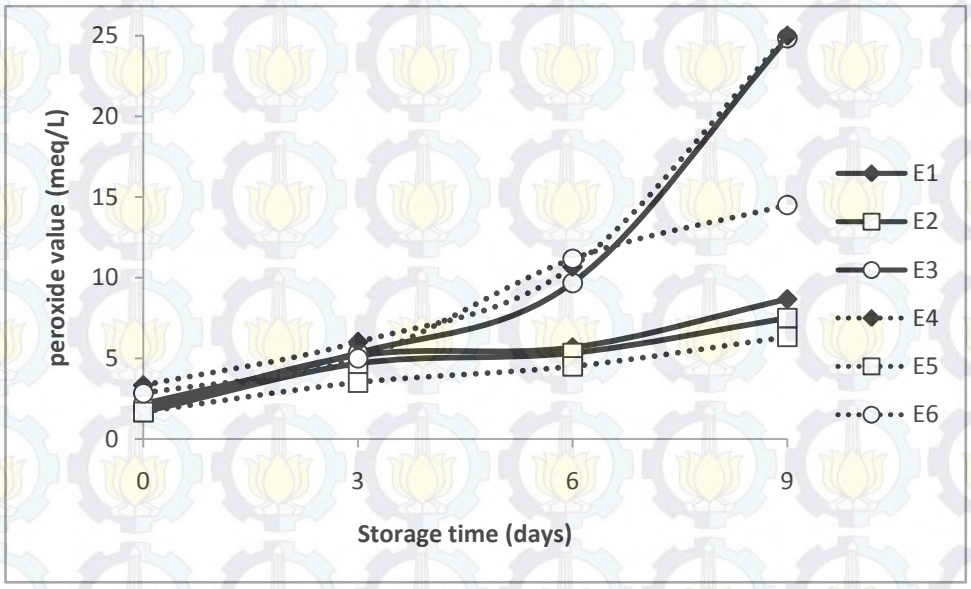

Figure 4.16 Peroxide valueof emulsions from RS and SPI

E_1= 7.5% SPI + 7.5% fish oil; E_2= 3.75% SPI + 3.75% RS + 7.5% fish oil; E_3= 3.75% SPI + 3.75% Hylon VII + 7.5% fish oil; E_4= 10% SPI + 5% fish oil; E_5= 5% SPI + 5% RS + 5% fish oil; E_6= 5% Hylon VII + 5% fish oil.

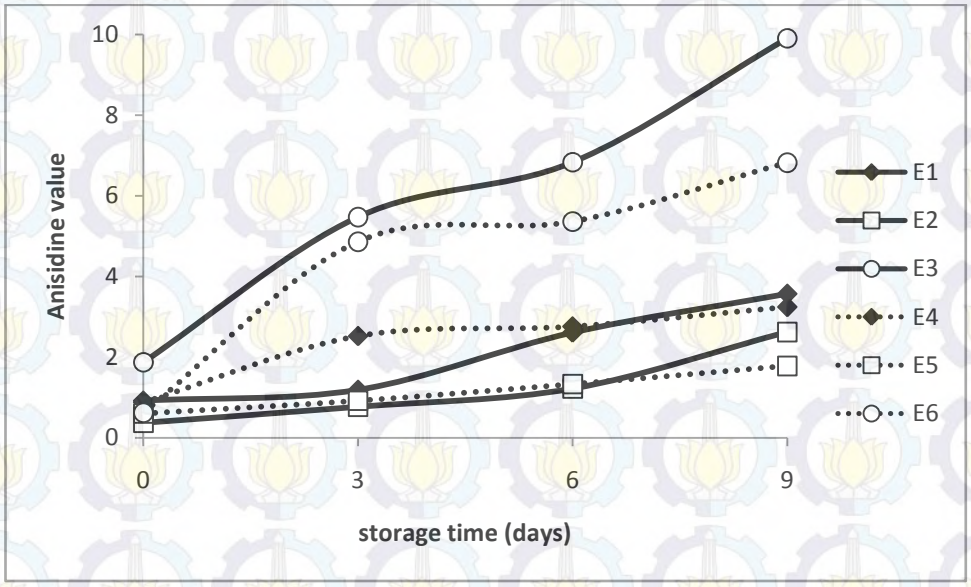

Figure 4.17 Anisidinevalueof emulsions from RS and Casein

E_1= 7.5% casein + 7.5% fish oil; E_2= 3.75% casein + 3.75% RS + 7.5% fish oil; E_3= 3.75% casein + 3.75% Hylon VII + 7.5% fish oil; E_4= 10% casein + 5% fish oil; E_5= 5% casein + 5% RS + 5% fish oil; E_6= 5% Hylon VII + 5% fish oil.

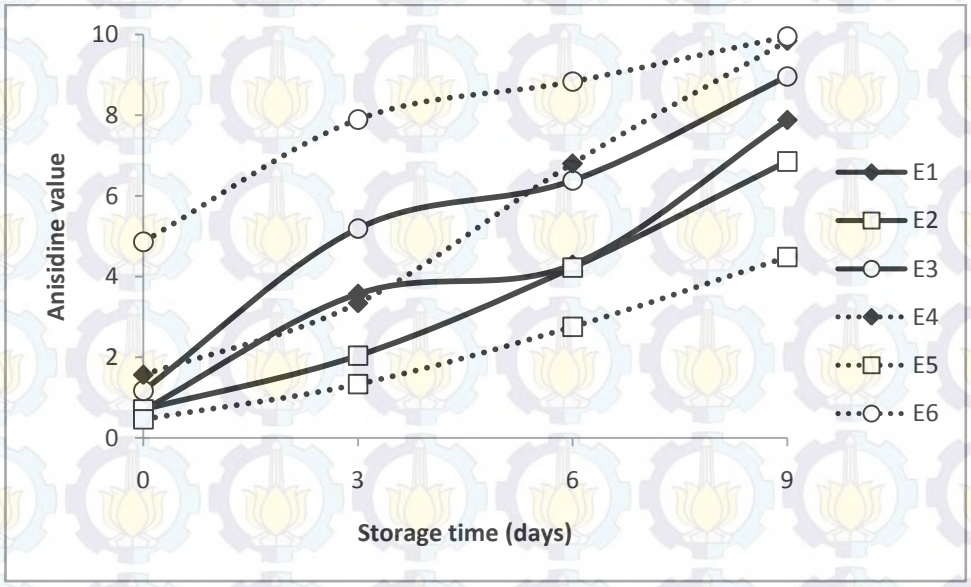

Figure 4.18 Anisidinevalueof emulsions from RS and SPI

E_1= 7.5% SPI + 7.5% fish oil; E_2= 3.75% SPI + 3.75% RS + 7.5% fish oil; E_3= 3.75% SPI + 3.75% Hylon VII + 7.5% fish oil; E_4= 10% SPI + 5% fish oil; E_5= 5% SPI + 5% RS + 5% fish oil; E_6= 5% Hylon VII + 5% fish oil.

CHAPTER 5
CONCLUSIONS AND RECOMMENDATIONS

1.1 Conclusions

1. Resistant starch type III (RS_3) was producted from sago starch by using variation of time and variation of citric acid concentration through lintnerization and lintnerization-autoclaving methods. Variation times (3; 6; 12 h) were not affect resistant starch production, but variation of citric acid concentrations (1; 1.5; 2 N) resulted different of RS contents. The highest RS content was obtained by using 2N of citric acid concentration through lintnerization-autoclaving method.

2. Physicochemicals of RS were compared by native sago starch, hydrolyzed starch by distilled water and lintnerized starch. Amylose content decreased after hydrolyzed by distilled water and lintnerization, but increasing by using lintnerization-autoclaving method. Protein and fat contents decreased after hydrolysis, but crude fiber content increasing, the highest value was obtained lintnerized-autoclaved starch. Lintnerized-autoclaved starch also exhibited the most resistant than other samples when hydrolyzed by α-amylase, pancreatic and pepsin. It also was proven with its microstructure analysis which had compact and rigid structure than others. UV/visible spectra showed the absorbance intensity decreased after lintnerization while increased when treated with hydrolysis by distlled water and lintnerization-autoclaving method. The RVA viscosity, swelling power and water holding capacity values reduced after all treatments. The lowest of these values were obtained lintnerized-autoclaved starch. Solubility at 95^{o}C increased after acid treatment.

3. Oil in water emulsions were also analyzed by mixture of RS and casein, compared also using mixture of RS and SPI, for comparison emulsions were made from Hylon VII using emulsifier (casein or SPI). Viscosities of emulsions from RS casein were lower (20.00 cP-31.99 cP) than those of RS-SPI (37.05 cP-52.07 cP). The highest L* value of RS-casein emulsions was

84.40, made from 5% casein+5% Hylon VII+ 5% fish oil while highest L* value of RS-SPI emulsion was 85.34, made from 7.5% SPI and 7.5% fish oil. Emulsion capacity and emulsion stability values were better gotten using RS-SPI than using RS-casein. The highest of emulsion capacity made from RS-casein was obtained 5.67% (3.75% casein+ 3.75 RS + 7.5% fish oil) while the highest that of RS-SPI was obtained 11.33% (5% SPI + 5% RS + 5% fish oil). The highest of emulsion stability value was gotten from mixture of emulsifier (Casein or SPI) and RS, but the higher value of emulsion stability of emulsion capacity was obtained when using mixture of RS and SPI (11.33%) than that of RS and casein (8.00%). For storage period, the lowest peroxide and anisidine values of mixture RS-SPI and RS-casein were resulted from 5% emulsifier (casein or SPI) + 5% RS + 5% fish oil, and the lowest percentage of these values was exhibited emulsion using mixture RS-SPI than RS-casein.

1.2 Recommendations

1. RS production can be researched using hydrolyzed by distilled water followed autoclaving.
2. RS can be used to functional bakery food, cereals and other foods because it contain diatary fibers which useful to body human.

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APPENDIX A

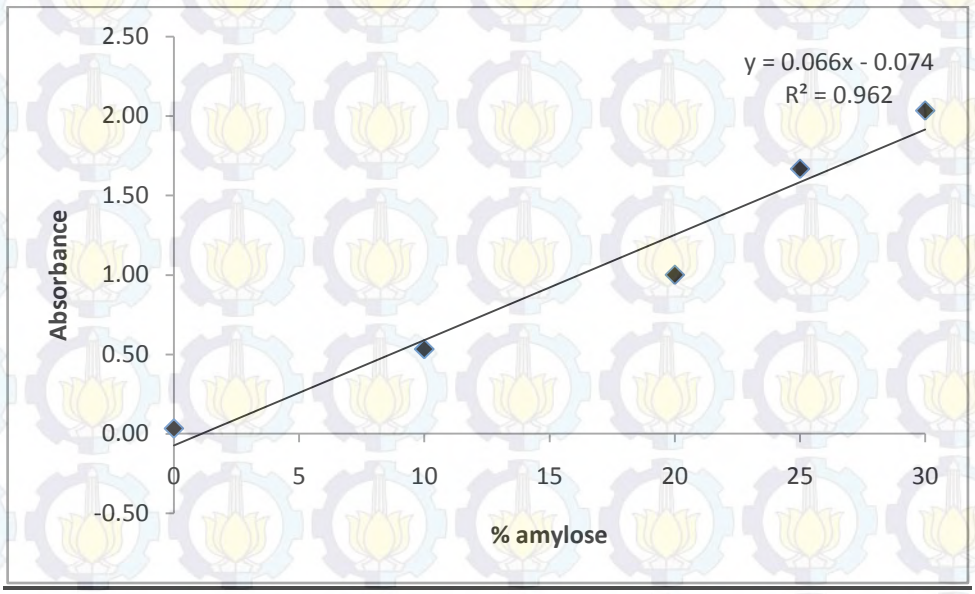

Figure 1. Standard curve for amylose determination by spectrophotometric assay

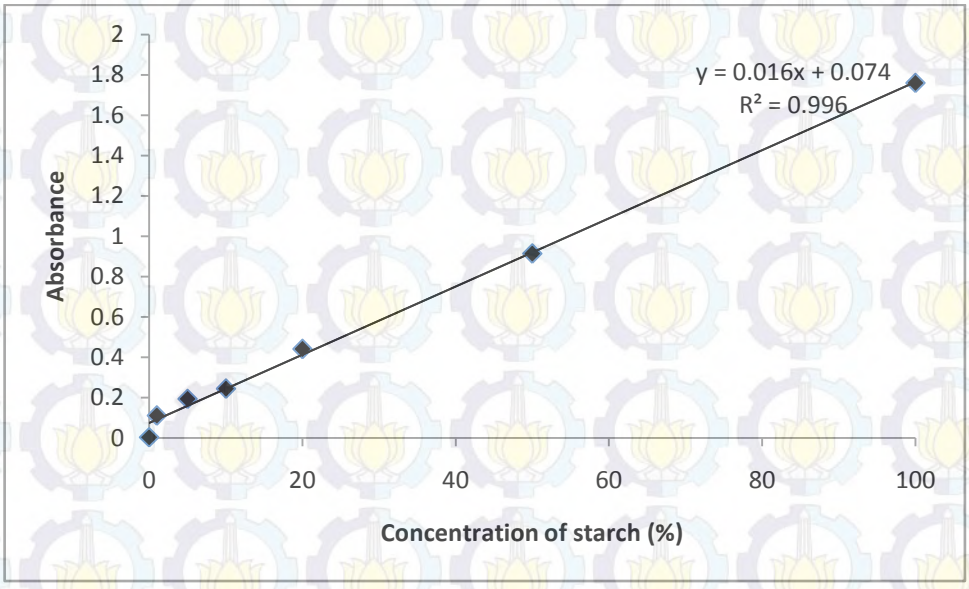

Figure 2. Standard curve for hydrolysis by α-Amylase

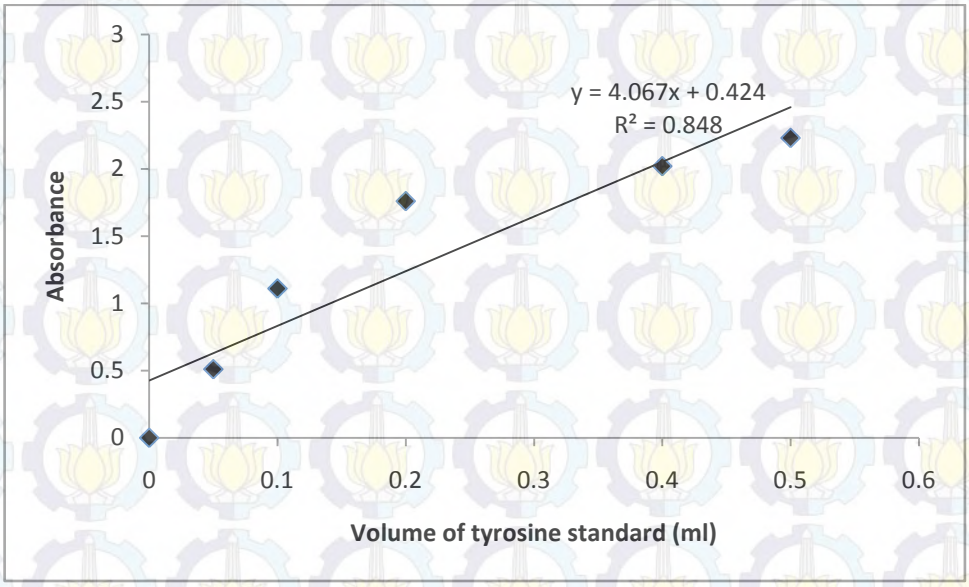

Figure 3. Standard curve for hydrolysis by pepsin

Table 1. Hydrolysis by Enzymes

Enzymes	Sample			
	Native	DW	L	LA
α-amylase (degraded starch, %)	1.34±0.002[b]	0.601±0.001[a]	0.618±0.21[a]	0.528±0.001[a]
Pancreatic (degree of hydrolysis, %)	6.667±0.578[c]	3.667±0.578[b]	3.333±0.578[b]	1.33±0.578[a]
Pepsin (Units/ml enzymes)	0.178±0.001[d]	0.169±0.002[c]	0.119±0.001[b]	0.089±0.001[a]

Data were mean and standard deviation of three determinations.
Values in the same column with different superscripts are statistically different ($p < 0.05$)
Native = sago starch; DW = hydrolyzed starch by distilled water; L= lintnerized starch; LA = lintnerized-autoclaved starch

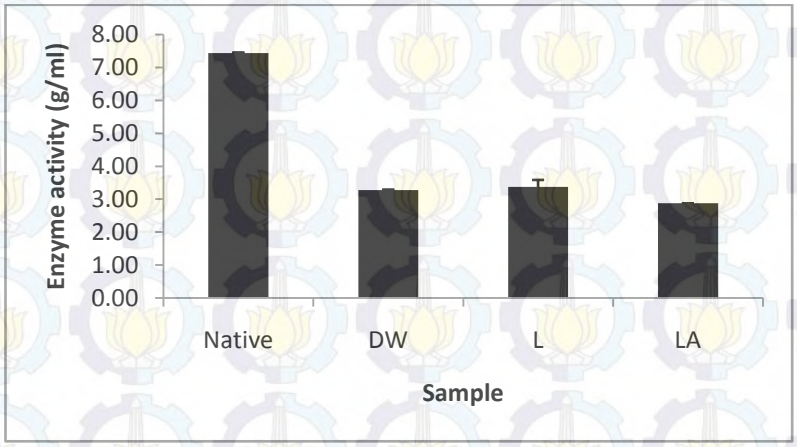

Figure 4. Hydrolysis by α-Amylase of native sago starch, hydrolyzed starch by distilled water (DW), lintnerized starch (L) and lintnerized-autoclaved starch (LA)

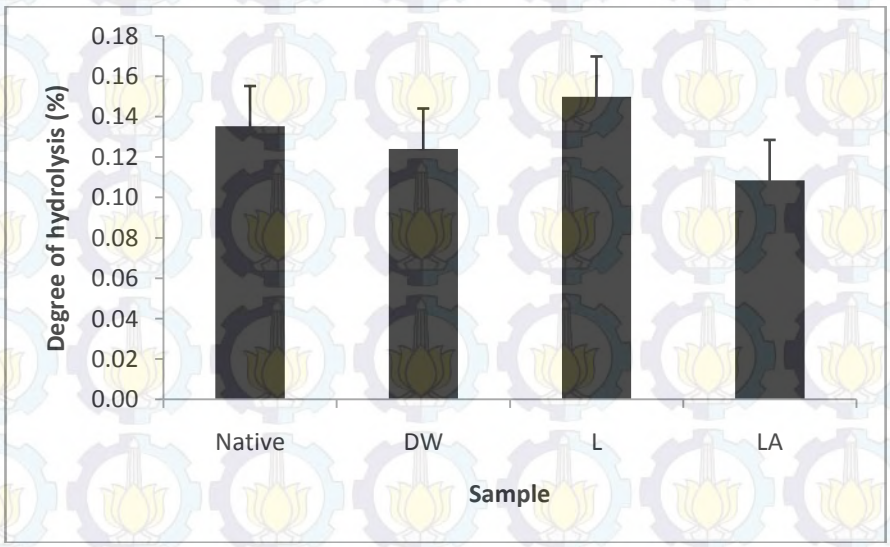

Figure 5. Hydrolysis by Pancreatic of native sago starch, hydrolyzed starch by distilled water (DW), lintnerized starch (L) and lintnerized-autoclaved starch (LA)

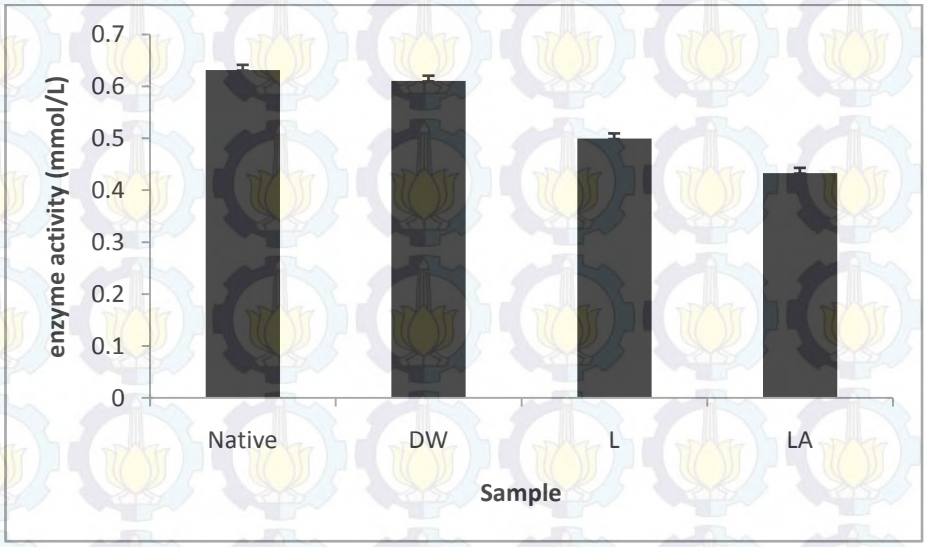

Figure 6. Hydrolysis by Pepsin of native sago starch, hydrolyzed starch by distilled water, lintnerized starch and lintnerized-autoclaved starch

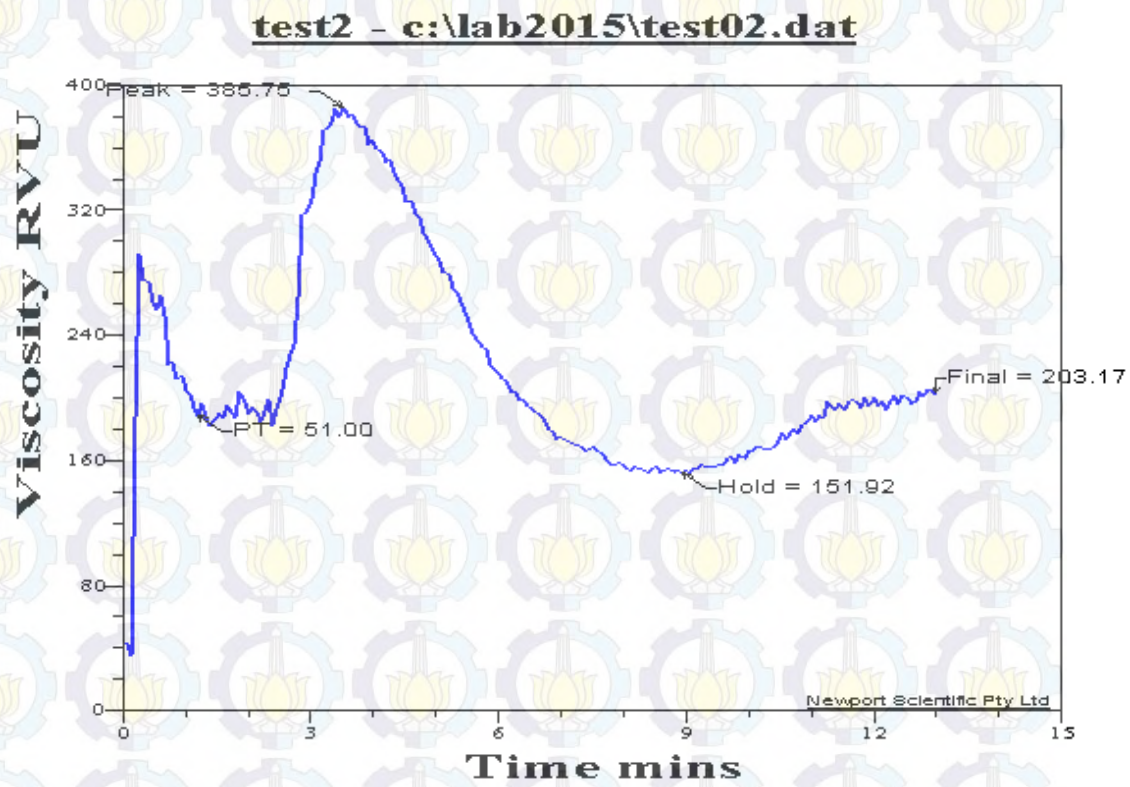

Figure 7. Pasting properties of native sago starch

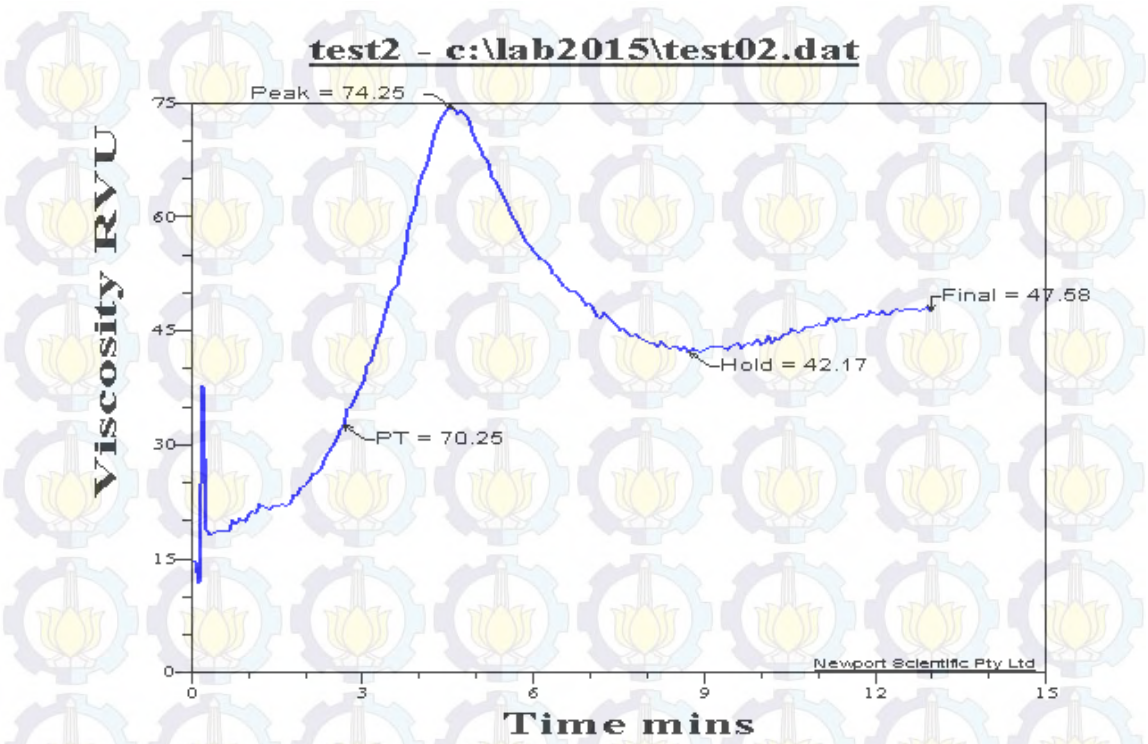

Figure 8. Pasting properties of hydrolyzed starch by distilled water

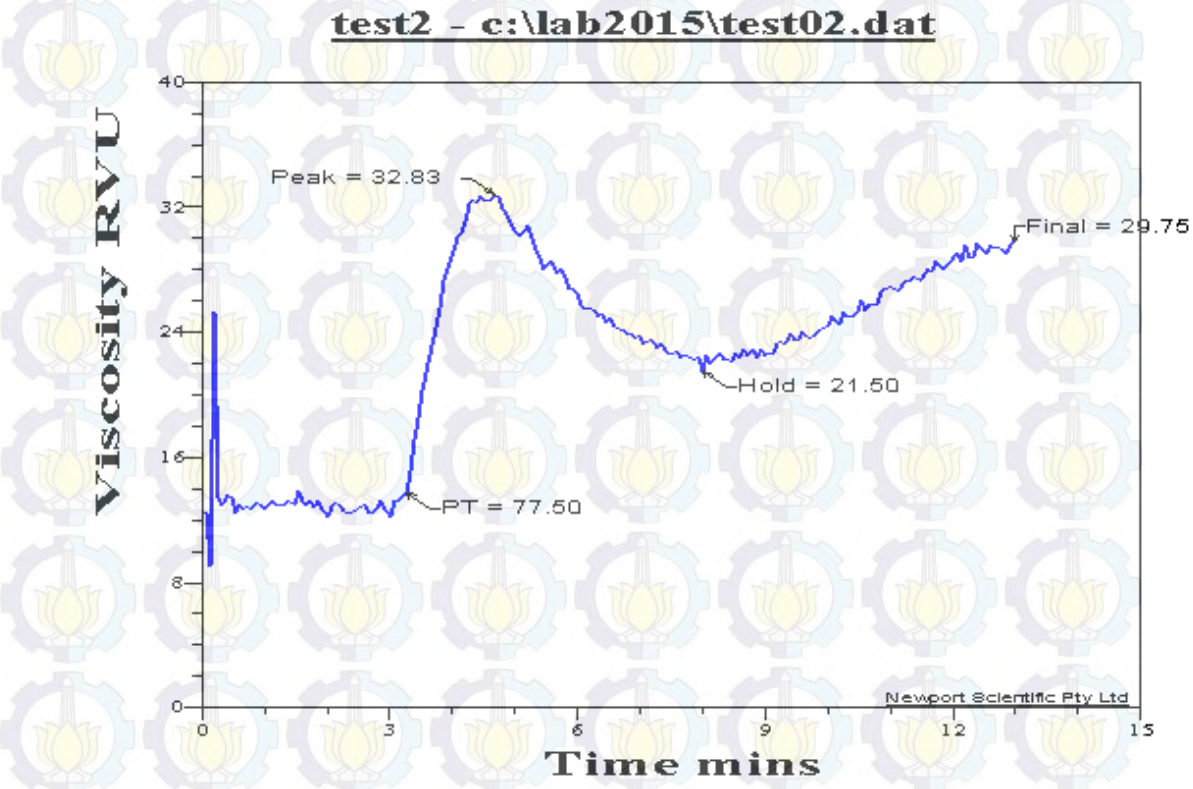

Figure 9. Pasting properties of lintnerized starch

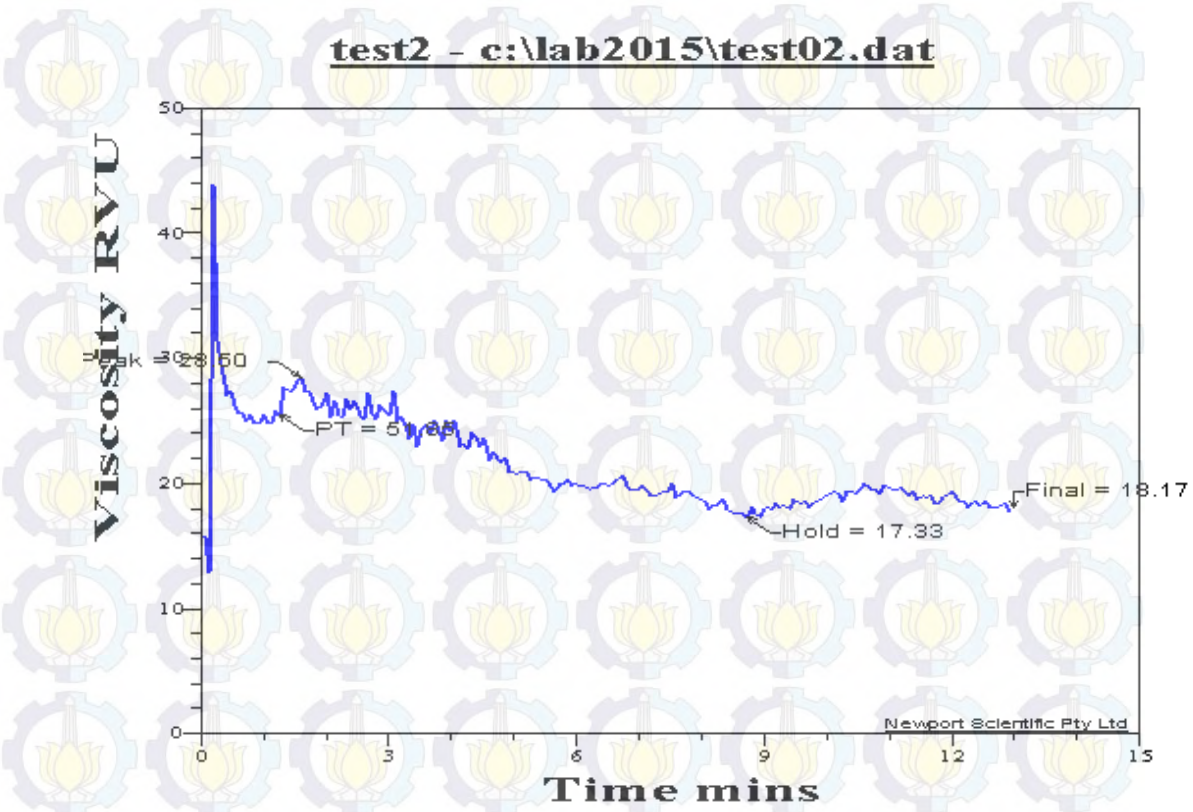

Figure 10. Pasting properties of lintnerized-autoclaved starch

Table 2. Percent swelling power of native sago starch, hydrolyzed starch by distilled water, lintnerized starch and lintnerized-autoclaved starch at different temperature

Temperature (^{o}C)	Native sago starch	Hydrolyzed starch by distilled water	Lintnerized starch	Lintnerized-autoclaved starch
40	2.36±0.02	6.68±0.11	3.34±0.03	4.47±0.03
50	2.38±0.03	9.67±0.01	3.64±0.04	4.72±0.03
60	3.16±0.01	10.17±0.09	4.12±0.03	4.79±0.02
70	3.52±0.01	11.64±0.02	9.85±0.03	7.75±0.02
80	15.37±0.01	11.75±0.02	10.65±0.01	8.36±0.01
90	21.30±0.02	12.79±0.06	19.39±0.01	11.52±0.01
95	27.62±0.01	16.69±0.01	21.71±0.01	12.37±0.01

Values are given as mean of triplicate determinations ± standard deviation

Table 3. Percent solubility of native sago starch, hydrolyzed starch by distilled water, lintnerized starch and lintnerized-autoclaved starch at different temperature

Temperature (^oC)	Native sago starch	Hydrolyzed starch by distilled water	Lintnerized starch	Lintnerized-autoclaved starch
40	1.00±0.001	11.33±0.002	4.00±0.0	10.67±0.006
50	2.00±0.0	21.67±0.006	8.00±0.0	13.33±0.006
60	2.67±0.012	28.33±0.012	12.33±0.012	15.00±0.0
70	11.67±0.012	31.33±0.006	36.33±0.006	33.33±0.006
80	13.67±0.012	38.00±0.0	38.67±0.012	42.67±0.012
90	44.67±0.032	49.67±0.006	64.00±0.0	44.67±0.006
95	52.00±0.017	54.33±0.012	64.67±0.012	52.67±0.015

Values are given as mean of triplicate determinations ± standard deviation

Table 4. Percent water holding capacity of native sago starch, hydrolyzed starch by distilled water, lintnerized starch and lintnerized-autoclaved starch at different temperature

Temperature (^oC)	Native sago starch	Hydrolyzed starch by distilled water	Lintnerized starch	Lintnerized-autoclaved starch
40	1.36±0.02	5.68±0.11	2.34±0.03	3.47±0.03
50	1.38±0.03	8.67±0.01	2.64±0.04	3.72±0.03
60	2.16±0.01	9.17±0.09	3.12±0.03	3.79±0.02
70	2.52±0.01	10.64±0.02	8.85±0.03	6.75±0.02
80	14.37±0.01	10.75±0.02	9.65±0.01	7.36±0.01
90	20.30±0.02	11.79±0.06	18.39±0.01	10.52±0.01
95	26.62±0.01	15.69±0.01	20.71±0.01	11.37±0.01

Values are given as mean of triplicate determinations ± standard deviation

Table 5. Peroxide value (meq/L) of different emulsions stored at 4^oC

Sample	Storage period (day)			
	0	3	6	9
1. RS+Casein				
E_1	5±0.08	5.33±0.05	6.33±0.13	8.17±0.09
E_2	5±0.14	5±0.08	6.67±0.2	7.67±0.09
E_3	6.5±0.08	9.5±0.14	10.83±0.1	24.33±0.09
E_4	6.5±0.08	8.83±0.05	11.33±0.2	24.33±0.12
E_5	3.67±0.05	4.5±0.08	5±0.2	6.67±0.05
E_6	9.17±0.09	11.67±0.12	12.5±0.22	15.83±0.3

2. RS+SPI				
E_1	1.83±0.06	5.17±0.15	5.67±0.21	8.67±0.15
E_2	1.67±0.06	4.67±0.06	5.33±0.15	7.5±0.17
E_3	2.17±0.12	5.33±0.06	9.67±0.15	24.83±0.15
E_4	3.33±0.05	6±0.17	10.67±0.12	25±0.1
E_5	1.67±0.12	3.5±0.3	4.5±0.1	6.33±0.06
E_6	2.83±0.11	5±0.2	11.17±0.31	14.5±0.2

Values are given as mean of triplicate determinations ± standard deviation
E_1= 7.5% emulsifier (casein or SPI) + 7.5% fish oil; E_2= 3.75% emulsifier + 3.75% RS + 7.5% fish oil; E_3= 3.75% emulsifier + 3.75% Hylon VII + 7.5% fish oil; E_4= 10% emulsifier + 5% fish oil; E_5= 5% SPI + 5% RS + 5% fish oil; E_6= 5% Hylon VII + 5% fish oil.

Table 6. Anisidine value of different emulsions stored at 4°C

Sample	Storage period (day)			
	0	3	6	9
1. RS+Casein				
E_1	0.92±0.01	1.19±0.01	2.61±0.03	3.57±0.03
E_2	0.37±0.003	0.77±0.01	1.22±0.014	2.62±0.02
E_3	1.87±0.02	5.47±0.1	6.83±0.06	9.9±0.1
E_4	0.88±0.01	2.52±0.02	2.76±0.03	3.24±0.03
E_5	0.6±0.01	0.92±0.08	1.33±0.01	1.78±0.02
E_6	0.62±0.01	4.86±0.04	5.360.06	6.82±0.06
2. RS+SPI				
E_1	0.69±0.006	3.57±0.03	4.29±0.04	7.88±0.07
E_2	0.7±0.008	2.04±0.02	4.22±0.04	6.85±0.06
E_3	1.17±0.014	5.19±0.05	6.38±0.06	8.96±0.08
E_4	1.56±0.015	3.34±0.03	6.8±0.06	9.84±0.09
E_5	0.46±0.01	1.33±0.01	2.75±0.03	4.48±0.04
E_6	4.86±0.05	7.89±0.08	8.83±0.08	9.95±0.1

Values are given as mean of triplicate determinations ± standard deviation
E_1= 7.5% emulsifier (casein or SPI) + 7.5% fish oil; E_2= 3.75% emulsifier + 3.75% RS + 7.5% fish oil; E_3= 3.75% emulsifier + 3.75% Hylon VII + 7.5% fish oil; E_4= 10% emulsifier + 5% fish oil; E_5= 5% SPI + 5% RS + 5% fish oil; E_6= 5% Hylon VII + 5% fish oil.

APPENDIX B

Analysis of variance (ANOVA) analyzed by SPSS

Table 1. Resistant starch value of lintnterized starch, lintnerized-autoclaved starch

Tests of Between-Subjects Effects

Dependent Variable: absorbance

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.000[a]	8	3.158E-005	8.702	.000
Intercept	.237	1	.237	65263.684	.000
time_of_hydrolysis	3.089E-005	2	1.544E-005	4.255	.031
citric_acid_concentration	.000	2	8.411E-005	23.173	.000
time_of_hydrolysis * citric_acid_concentration	5.356E-005	4	1.339E-005	3.689	.023
Error	6.533E-005	18	3.630E-006		
Total	.237	27			
Corrected Total	.000	26			

a. R Squared = .795 (Adjusted R Squared = .703)

Table 2. Chemical composition of native sago starch, hydrolyzed starch by distilled water, lintnterized starch, lintnerized-autoclaved starch

		Sum of Squares	df	Mean Square	F	Sig.
Amylose	Between Groups	.003	3	.001	1102.533	.000
	Within Groups	.000	8	.000		
	Total	.003	11			
Fiber	Between Groups	.003	3	.001	1.025	.431
	Within Groups	.007	8	.001		
	Total	.009	11			
Moisture	Between Groups	.080	3	.027	10.742	.004
	Within Groups	.020	8	.002		
	Total	.100	11			
Ash	Between Groups	.006	3	.002	3626.812	.000
	Within Groups	.000	8	.000		
	Total	.006	11			
Protein	Between Groups	.403	3	.134	80.667	.000
	Within Groups	.013	8	.002		
	Total	.417	11			

Fat	Between Groups	.001	3	.000	17.333	.001
	Within Groups	.000	8	.000		
	Total	.001	11			

Table 3. Pasting properties of native sago starch, hydrolyzed starch by distilled water, lintnterized starch, lintnerized-autoclaved starch

		Sum of Squares	df	Mean Square	F	Sig.
Peak	Between Groups	306333.084	3	102111.028	310.195	.000
	Within Groups	2633.464	8	329.183		
	Total	308966.548	11			
Through	Between Groups	34360.815	3	11453.605	751.835	.000
	Within Groups	121.874	8	15.234		
	Total	34482.688	11			
Break - down	Between Groups	136166.890	3	45388.963	138.425	.000
	Within Groups	2623.172	8	327.896		
	Total	138790.062	11			
Final	Between Groups	66150.423	3	22050.141	768.923	.000
	Within Groups	229.413	8	28.677		
	Total	66379.836	11			
Setback	Between Groups	5241.855	3	1747.285	184.259	.000
	Within Groups	75.862	8	9.483		
	Total	5317.717	11			
Peak-time	Between Groups	16.585	3	5.528	2.588	.126
	Within Groups	17.092	8	2.137		
	Total	33.678	11			
Pasting_ temp	Between Groups	505.084	1	505.084	105.153	.001
	Within Groups	19.213	4	4.803		
	Total	524.297	5			

Table 4. ANOVA of Emulsion capacity (EC) and Emulsion stability (ES) of RS-SPI emulsions

		Sum of Squares	df	Mean Square	F	Sig.
EC	Between Groups	.258	5	.052	4.640	.014
	Within Groups	.133	12	.011		
	Total	.391	17			
ES	Between Groups	.203	5	.041	3.174	.047
	Within Groups	.153	12	.013		
	Total	.356	17			

Table 5. ANOVA of Emulsion capacity (EC) and Emulsion stability (ES) of RS-Casein emulsions

		Sum of Squares	df	Mean Square	F	Sig.
EC	Between Groups	.225	5	.045	6.750	.003
	Within Groups	.080	12	.007		
	Total	.305	17			
ES	Between Groups	.565	5	.113	13.560	.000
	Within Groups	.100	12	.008		
	Total	.665	17			

Table 6. ANOVA of color spectra and viscosity of RS-Casein emulsions

		Sum of Squares	df	Mean Square	F	Sig.
L	Between Groups	177.510	5	35.502	2147.293	.000
	Within Groups	.198	12	.017		
	Total	177.708	17			
a*	Between Groups	1.840	5	.368	49.167	.000
	Within Groups	.090	12	.007		
	Total	1.929	17			
b*	Between Groups	21.874	5	4.375	80.075	.000
	Within Groups	.656	12	.055		
	Total	22.529	17			
Viscosity	Between Groups	1419.296	5	283.859	906.432	.000
	Within Groups	3.758	12	.313		
	Total	1423.054	17			

Table 7. ANOVA of color spectra and viscosity of RS-SPI emulsions

		Sum of Squares	df	Mean Square	F	Sig.
L	Between Groups	18.963	5	3.793	63.933	.000
	Within Groups	.712	12	.059		
	Total	19.675	17			
a*	Between Groups	1.449	5	.290	71.660	.000

	Within Groups	.049	12	.004		
	Total	1.498	17			
b*	Between Groups	12.891	5	2.578	12.502	.000
	Within Groups	2.475	12	.206		
	Total	15.366	17			
viscosity	Between Groups	375.830	5	75.166	72.595	.000
	Within Groups	12.425	12	1.035		
	Total	388.255	17			

BIOGRAPHY

The author's full name is Wiwit Sri Werdi Pratiwi and she was born in Pamekasan, March 30, 1991. She is the first child of three siblings. The author has formal education is in SDN Jung Cang-Cang 1, SMPN 1 Pamekasan, SMAN 1 Pamekasan, Bachelor of Chemistry in Institute of Technology Sepuluh Nopember, Surabaya and the last was included in Joint Degree Program between ITS (Chemistry) and Asian Institute of Technology-Thailand (Food Engineering and Bioprocess Technology) in 2013. During the studies, the author was active in non-academic activity as researches. The author can be reached at wiwit.swiper@gmail.com.